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SCIENTIFIC PROCEEDINGS

1 (1961)

An oxyhæmoglobinometer for the clinical measurement of cyanosis.

By P. J. FLAGG (by invitation)

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Cyanosis occurs as a local or general manifestation of oxygen unsaturation. Localized cyanosis, such as is seen in the blue lips of bathers experiencing a temporary superficial vaso constriction for heat preservation, or, that which is seen as the result of gravity, (Trendelenburg posture) constriction by Tourniquet, etc., is quite a different matter from the generalized cyanosis observed in patients with warm perspiring skin free from all effects of gravity. A simple capillary stasis in a localized region usually accounts for the former condition; while the latter implies a systemic sub-oxygenation of far reaching significance. While cyanosis is seen in diseases of the heart and lungs, in ascent to high altitudes and in various forms of chemical poisoning, there is, perhaps, no condition in which the extremes may be so readily studied as during the administration of a general anæsthetic.

Some eight years ago, while teaching anæsthesia, the writer became impressed with the need of a clinical measure of cyanosis.

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The general term, or, even its gradations spoken of as marked, moderate, or a mild degree of cyanosis, conveyed no accurate idea of the condition under observation. Furthermore, it was noted that when an observer had been with a patient for some time, his appreciation or registration of a color witnessed became less acute. It is a common occurrence for a spectator to enter an operating room and be struck by the patient's cyanosis, the condition having been imperceptibly increased under the color wearied gaze of the anæsthetist. A standard measure of cyanosis, therefore, serves two purposes. It provides data relative to the degree of cyanosis present and provides a criterion for the color wearied vision of the constant observer.

That there has been a general failure to meet the demand for a measure of cyanosis is possibly due to a rather unique and widely accepted impression that where there is cyanosis there must be an increased tension of carbon dioxide. This accidental association of carbon dioxide and cyanosis has so complicated the matter of measuring cyanosis that, to the average medical mind, the solution was quite out of the question. To brush away the cobwebs of carbon dioxide as an element affecting the color of the blood is to reduce the problem to its simplest terms.

The observations which follow are based upon the assumption that the color of the blood, where the hæmoglobin is approximately normal, is directly dependent upon the amount of oxygen present as oxyhæmoglobin. Complete oxygen saturation (0 no cyanosis) representing one extreme; complete oxygen unsaturation (100 per cent. or complete cyanosis) representing the other.

Through the courtesy of Dr. Van Slyke of the Rockefeller Institute, numerous specimens of venous blood taken from the distal portions of ligated veins, after 5, 10, 15, 20 minutes stagnation, were subjected to quantitative analysis. The longest exposure resulted in oxygen unsaturation (cyanosis) of not more than 30 per cent. An attempt to secure a completely unsaturated specimen from an amputated leg also proved a failure. It became evident that it would not be possible to secure a completely unsaturated specimen from the blood while in the blood vessels, the oxygen tension being probably upheld by a flow of oxygen from the tissues, and possibly from atmospheric absorption from the mucous membranes.

At the suggestion of Dr. Van Slyke a specimen of oxalated blood was artificially unsaturated by passing hydrogen gas over it. It was found that the lower percentages of oxygen were displaced with difficulty. Finally, a specimen exposed to hydrogen gas for an hour and 15 minutes was found by quantitative analysis to be completely unsaturated (100 per cent. cyanosis). Before submitting this specimen for examination, the mass color was carefully matched by a color expert and recorded in nonfading ink. Complete saturation (0 cyanosis) was easily arrived at by passing oxygen gas through a specimen of oxalated blood. The two extremes of the mass color of the blood thus secured formed the basis for the intermediate percentages. This scale based upon the mass color of the circulating blood provides a fixed and optically accurate means of determining the percentage of specimens collected from blood vessels under the usual airtight precautions. It also offers a measure for the degree of oxygenation of blood escaping from a fresh incision when the skin and mucous membranes are not available for estimation, as for example, in operations for brain tumors, glands of the neck, thyroidectomies, etc.

The basic extremes representing the actual mass color of the blood having been secured, the next problem was to so modify this scale that the resultant color would faithfully represent the actual color seen in the nails, skin and mucous membranes.

It soon became apparent that the skin color depended upon the volume of the capillary circulation as well as upon the quality, hue or oxygen percentage of this circulation, *i.e.,* the normal individual at rest shows a delicate skin color whose variations are read with difficulty. The same individual after physical exertion or under anæsthesia will fall into a totally different range of color whose variations are much more clearly defined. While convinced that the skin and mucous membrane colors were fundamentally dependent upon basic blood scale already arrived at, the writer, nevertheless, essayed a determination of actual skin color under anæsthesia by enlisting the assistance of a color expert. What was his chagrin to find that it was not possible to accurately match the colors under observation? For there appeared to be a constant undulation of color value due to the respiratory complications of anæsthesia. Had it not been the

writer's good fortune to become acquainted with the Munsell system of color notation, it is quite likely that the end achieved could not have been accomplished. This system offers a conception of color in three dimensions and provides means of measuring accurately any color under observation.

Unknown colors are arrived at by spinning disks containing segments of colors of known hue, values and chroma. The optical mixtures resulting are unvarying and may be actually matched by fast colors. Employing the original blood scale as a base a color segment was added, which, on being rotated, yielded a result closely corresponding to fingernail and mucous membrane color. By varying the amount of this dilutant, various ranges were secured, representing the anaemic normal and plethoric types. The scales so made, while corresponding fairly well to the color of the skin, lacked brilliancy, and left much to be desired. While seeking a reason for this discrepancy, an interesting fact was noted:

It was observed that the color of completely oxygenated blood *en masse* was in accordance with previous findings of a brilliant claret hue. A drop presented the same color. If, however, this drop was spread out to represent a thin film, the color changed to a brick red, and, if further diluted, to a reddish yellow. The thin film of brick red representing the capillary circulation was then spun with the dilutant before used and the desired brilliancy was secured. The resulting scale offers gradations so close to the skin color that the line of demarkation can scarcely be seen.

The hæmoxometer (oxyhæmoglobinometer) consists of a series of scales representing the oxyhæmoglobin content of the blood as seen in the actual mass blood, mucous membrane and the finger nail, or skin of the normal plethoric and anæmic individual. Each step of the scale is arrived at by a separate process, mathematically and optically accurate. The skin is read at the lobe of the ear, the mucous membrane at the bucal aspect of the lips or the palpebral conjunctiva. The finger nails read, directly, represent about the same opacity as the skin.

By employing this device, one may determine not only the approximate oxyhæmoglobin content of the blood, but the volume as well. By observing into what range the color falls, the super-

ficial circulation may be checked up and variations in blood pressure may be anticipated.

CONCLUSIONS.

The need of a simple clinical measure of cyanosis has long been felt, not only by surgeons and anæsthetists, but by physicians who have under their care pulmonary and cardiac cases.

It is contended that sub-oxydation or oxygen unsaturation of the total circulating blood is abnormal and imperils the life of the patient.

There appears to be a large unexplored field in the physiological limits of oxygen unsaturation. This field includes obstetrics.

By simple laboratory methods the two extremes of complete cyanosis and complete oxygenation have been secured.

The exact color value of these extremes has been permanently recorded by the help of the Munsell system of color notation.

Intermediate gradations of these extremes have been secured by mechanical means, so that a scale is available.

The resulting scale, the hæmoxometer (oxyhæmoglobinometer) is a positive basic measure of the oxyhæmoglobin content of the blood under observation. The opacity presented by the finger nail and mucous membrane has been studied and closely duplicated.

The hæmoxometer is offered as a simple clinical measure of oxygen unsaturation (cyanosis), *basically correct,* in practice, *approximate.*

In submitting this original device, the writer is convinced that it has a sphere of usefulness scarcely second to that of the hæmoglobinometer.

2 (1962)

Allergy to cow's milk in infants with severe malnutrition.

By OSCAR M. SCHLOSS and ARTHUR ANDERSON

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There is much evidence to indicate that under certain conditions the intestinal tract of infants is permeable to slightly altered or unaltered protein.

This has been demonstrated to be true of egg protein by Lust, Hyashi, Schloss and Worthen and others and for the proteins of cow's milk by Modigliani and Benini and Schloss and Worthen. These investigations were based on the demonstration of the protein in the blood or urine by means of precipitin tests or anaphylactic tests on guinea pigs. Such passage of unaltered or partially digested protein is especially apt to occur in malnourished infants or in those suffering from diarrhea.

The investigations cited all show the possibility of foreign protein entering the blood stream through the intestinal wall. If this were a frequent occurrence it would seem of interest to determine whether the entrance of foreign protein under these conditions provoked the production of antibodies. This question is probably of more than academic importance. Moro found precipitines to cow's milk in the blood of 2 out of 24 atrophic infants examined post mortem. Bauer demonstrated precipitines in the blood of 4 atrophic infants.

During the past two years we have conducted investigations on infants with nutritional disorders to determine the frequency with which antibodies to milk occur in the blood.

Precipitin tests for antibody were done as follows: To 1 c.c. of milk dilutions of 1-100, 1-500 and 1-1000 was added 0.1 c.c. of the serum to be tested. The tests were read after incubation for 1 hour at 37.5° C, the tubes were then kept on ice for 24 hours and read a second time.

In the earlier tests controls of normal human serum and normal rabbit or sheep serum were used. These tests were uniformly negative and were omitted in the later tests. Positive controls were made by using anti-milk rabbit serum. Breast milk in similar dilutions to the cow's milk was used as a control in some of the tests. Control tests, using the serum from the patient investigated diluted with normal saline, were used throughout.

The tests for anaphylactic antibody were conducted as follows: Three c.c. of citrated blood were injected into the peritoneal cavity of a 200 to 300 gram guinea pig. Twenty-four hours later 0.5 to 0.75 c.c of fat free milk were injected intravenously.

Tests for antigen were made by adding 0.1 c.c of anti-milk rabbit serum too .1 c.c. of the serum to be tested diluted to 1 c.c. with normal saline solution.

The following control tests were used:

(1) One-tenth c.c. of the serum tested was diluted to 1 c.c. with normal saline.

(2) One-tenth c.c. of the anti-serum was diluted to 1 c.c. with saline.

(3) One-tenth c.c. of normal rabbit serum was added to 0.1 c.c. of the serum tested diluted to 1 c.c. with saline.

(4) One-tenth c.c. of normal human serum was diluted to 1 c.c. with normal saline solution and 0.1 c.c. of the anti-milk serum added.

The controls were uniformly negative and all were not used in each series of tests. In the later tests only 1 and 2 were used.

The anaphylactic tests for antigen were made by injecting 3 c.c. of the citrated blood into the peritoneal cavity of a 200 to 300 gram guinea pig. Three weeks later an intoxicating dose of 0.5 to 0.75 c.c. of milk was injected intravenously.

Observations were conducted on 37 patients with athrepsia and in 24 the precipitine tests were positive on at least one occasion. In many cases the test was repeated from one to eight times.

In 13 of the cases with positive precipitine tests the attempt was made passively to sensitize guinea pigs by injections of the patients' blood. The results were positive in 8 cases. The results were considered positive only when the animals developed very severe symptoms. When the symptoms were slight or open to any question the experiment was considered negative or was excluded. In the tests considered positive the animals developed marked dyspnea, convulsive movements or paralysis and in most instances died within 15 minutes after the injection. The necropsies disclosed marked distension of the lungs.

Throughout the investigation many control tests were made. Intravenous injections of milk in the quantities used caused no symptoms in normal guinea pigs. All of the positive experiments on animals were in cases in which the blood showed precipitines for cow's milk. In no instance did the blood of infants not showing precipitines for cow's milk passively sensitize guinea

pigs to cow's milk. In 33 cases with negative precipitine tests attempts passively to sensitize guinea pigs with the patient's blood were negative.

In a number of the patients observations were made over a period of from 2 to 4 months. It was found that the presence of precipitines varied from time to time. Thus far our observations have been too few to correlate the presence or absence of precipitines with the clinical condition of the patient. In four patients, however, who were observed over a long period, the precipitine reaction was absent during periods when the infant was losing weight or when the weight was stationary and reappeared when the patient improved. With further improvement the precipitines disappeared entirely. This question, however, must be studied further before any conclusions can be drawn.

A group of 30 infants suffering from a mild or moderate grade of malnutrition or convalescent from acute infections were studied. In 25 cases the tests for precipitines to cow's milk were negative and in 5 positive. In two of the positive cases passive sensitization tests on guinea pigs were tried. Both were positive. Fifteen tests on older children or adults were negative. The blood of eleven new born infants (placental blood) was examined and was negative in all.

Tests for milk protein in the blood were made in 27 cases of severe diarrhea by the precipitine reaction and also by anaphylactic tests on guinea pigs. All of the patients were receiving cow's milk as food. In six cases the precipitine test was positive. In 17 cases the anaphylactic tests on guinea pigs were positive. In 10 control tests on athreptic infants both the precipitine and anaphylactic tests for antigen were negative. These results confirm the work of Lust, Schloss, and Worthen, and others, in showing that in diarrhea there is a tendency for the intestinal tract to be permeable to protein. In this connection tests for precipitine to cow's milk on 13 infants suffering from diarrhea or convalescent from diarrhea are of interest. In all of these cases precipitine was present. Passive sensitization tests on guinea pigs were made in 8 cases and were positive in 3.

These observations seem to indicate that the blood of athreptic infants may show the presence of reaction bodies to cow's milk both by the presence of a precipitine to cow's milk protein and by the fact that the blood of the infant can passively sensitize guinea pigs to cow's milk.

Whether the presence of these reaction bodies bears a causative relation to the clinical condition we do not feel in a position to discuss with assurance at the present time. That this may be true seems quite possible and is in accord with many clinical observations. On the other hand, the presence in the blood of athreptic infants of reaction bodies to cow's milk protein may be due merely to the fact that the intestinal tract of such infants has permitted the passage of cow's milk protein into the blood stream, thereby causing antibody formation, and may not be of etiological importance. Observations are being conducted at present which may throw some light on this question.

3 (1963)

Carbon assimilation and respiration of autotrophic bacteria[1]

By SELMAN A. WAKSMAN and ROBERT L. STARKEY

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The autotrophic bacteria, or those bacteria which are capable of assimilating carbon dioxide chemosynthetically utilizing for that purpose the energy obtained from the oxidation of simple inorganic substances, range from obligate to facultative forms. In other words they range from those which are capable of obtaining their energy only from the oxidation of specific inorganic substances but from no other source to those that can exist autotrophically and heterotrophically; the latter can obtain their energy both from inorganic sources and, lacking these, also from the oxidation of organic compounds. The nitrite, nitrate and certain sulfur bacteria are the only strict autotrophic forms, while all the others, including various sulfur bacteria,

the methane, hydrogen and particularly iron bacteria belong to the second group.

Respiration in the case of autotrophic bacteria is different from that of the heterotrophic forms; it consists in taking in carbon dioxide and giving out oxygen, at the same time consuming large quantities of oxygen for purposes of oxidation which yields the energy necessary for the activities of the organisms. Meyerhof was the first to demonstrate conclusively that the nitrite and nitrate forming bacteria obtain their carbon only from the carbon dioxide of the atmosphere or in the form of bicarbonate in solution. To demonstrate the carbon assimilation by the sulfur-oxidizing organism, *Sulfomonas thiooxidans,* the following procedure was followed: A medium consisting of 0.2 gm. $(NH_4)_2SO_4$, 3 gm. KH_2PO_4, 0.5 gm. $MgSO_4$, 0.25 gm. $CaCl_2$, 0.01 gm. $FeSO_4$ and 10 gm. of elementary sulfur per 1000 c.c. of distilled water, placed in 100 c.c. portions in 250 c.c. Erlenmeyer flasks was sterilized and inoculated in the usual manner. To some of the flasks 1 per cent. of dextrose was added, or 0.1 per cent. of $NaHCO_3$, or 0.1 per cent. $NaHCO_3$ and sufficient $\dfrac{N}{1} H_3PO_4$ to neutralize the excess alkalinity. The flasks were divided into two series; (1) placed in the incubator kept at constant temperature and (2) placed under bell jars, with a carbon-dioxide free atmosphere. The air passing through the jars was drawn through a soda-lime tube and bottles containing 50 per cent. solution of KOH. The jars were sealed on to a bench in the same incubator (at 28° C) with the first series of flasks. At the end of 7 days, the hydrogen-ion concentration and titratable acidity (for 10 c.c.) of the cultures were determined. These serve as an index of the amount of sulfur oxidized.

Treatment	Atmosphere			
	Ordinary		CO_2 free	
	Final P_H	Titre	P_H	Titre
Regular medium—Control	4.2	2.20	4.2	2.20
Regular medium—Inoculated	1.2	12.15	3.8	2.25
1 per cent. Dextrose—Control	3.0	2.20	3.0	2.2
1 per cent. Dextrose—Inoculated	1.2-	13.15	2.8	2.33
0.1 per cent. $NaHCO_3$—Control	6.6	1.3	6.6	1.3
0.1 per cent. $NaHCO_3$—Inoculated	5.4	2.0	6.0	1.75
0.1 per cent. $NaHCO_2$ + H_3PO_4—Control	6.2	2.2	6.2	2.2
0.1 per cent. $NaHCO_2$ + H_3PO_4—Inoculated	1.5	9.3	5.3	2.5

No growth took place in the carbon-dioxide free atmosphere both in the absence and presence of dextrose. The slight increase in acidity of the inoculated culture is due to the inoculum introduced (3 drops of culture in 100 c.c. of medium). However, in the presence of the bicarbonate, a slight amount of growth took place even in the CO_2-free atmosphere. The relatively small growth made in the presence of the bicarbonate is due to the change in reaction of the medium, the organism having its optimum at P_H 2.0 to 5.0 (1.0-5.6).

For studying the respiration of bacteria, the methods of Osterhout[2] and Meyerhof[3] are available. The former is not very suitable for the study of the autotrophic bacteria, since the organisms consume carbon dioxide and produce acids which rapidly change the reaction of the medium. The method used by Meyerhof was found more suitable. By this method we measure the amount of change in the concentration of substratum produced in a definite period of time, as a result of the energy utilization by the organism. We can thus differentiate between the growth and respiration processes.

Sulf. thiooxidans was grown in the above medium till the maximum rate of growth was attained, as determined from the

2 Osterhout, W. J. V., *J. Gen. Physiol.*, 1918, i, 171.

3 Meyerhof, O., *Arch. Ges. Physiol.*, 1916, clxiv, 353; clxv, 229; clxvi, 240.

autocatalytic curve of growth.   This took place at the end of
the fourth to the fifth day of incubation.   The culture was then
filtered through filter paper to get it free from sulfur.   50 c.c.
portions of the uniform culture were then placed in small flasks
(100 c.c. Erlenmeyer), to each of which 1 gram of elementary
sulfur was added.   The stimulating or repressive agents were
then added and the flasks placed in the incubator and kept at
30°C for about 20 hours.   The change in hydrogen-ion con-
centration, titration or sulfate content during that period in-
dicates the amount of sulfur oxidized and can serve as an in-
dex of oxygen consumption or carbon dioxide assimilation.   One
is particularly justified in doing that, since the ratio between
the sulfur oxidized and carbon dioxide assimilated is constant
under the conditions of the experiment, as pointed elsewhere.
The change in titration, using phenolphthalein as an indicator,
is the simplest index of the respiration of the culture, since at
the very high acid concentration at which the organism is active,
particularly in well buffered media, the change in hydrogen-ion
concentration is not a sensitive index.

If the normal rate of respiration is taken as a 100, the respira-
tion of the organism as influenced by various treatments is as
follows:

INFLUENCE OF GASES, NITRATES AND ORGANIC SUBSTANCES UPON THE RESPIRATION OF SULF. THIOOXIDANS

Control				100
$(NH_4)_2SO_4$	0.025	molar		108
$(NH_4)_2SO_4$	0.050	molar		112
$(NH_4)_2SO_4$	0.100	molar		112
$(NH_4)_2SO_4$	0.250	molar		108
$NaNO_3$	0.025	molar		29.2
$NaNO_3$	0.050	molar		12.5
$NaNO_3$	0.100	molar		0
$NaNO_3$	0.250	molar		0
$Ca(NO_3)_2$	0.025	molar		27.3
$Ca(NO_3)_2$	0.050	molar		8.5
$Ca(NO_3)_2$	0.100	molar		10.0
$Ca(NO_3)_2$	0.250	molar		0
$NaNO_3+(NH_4)_2SO_4$	0.0125	molar	each	62.5
$NaNO_3+(NH_4)_2SO_4$	0.025	molar	each	25.0
$NaNO_3+(NH_4)_2SO_4$	0.050	molar	each	16.5
$NaNO_3+(NH_4)_2SO_4$	0.125	molar	each	0
KNO_3	0.005	molar		71
KNO_3	0.010	molar		57
KNO_3	0.020	molar		43
KNO_3	0.050	molar		21
$Mg(NO_3)_2$	0.050	molar		16
$NaCN$	0.0004	molar		0

Peptone	0.2 per cent.	50
Peptone	0.4 per cent.	4.2
Peptone	1.0 per cent.	0
Peptone	2.0 per cent.	0
Dextrose	0.25 per cent.	133
Dextrose	1.00 per cent.	120
Tyrosin	0.1 per cent.	64
Glycocoll	0.1 per cent.	100
Glycocoll	0.5 per cent.	45
Urea	0.1 per cent.	100
Urea	0.5 per cent.	57
Ethyl Alcohol	0.1 per cent.	54
Ethyl Alcohol	0.3 per cent.	46
Amyl Alcohol	0.1 per cent.	43
Amyl Alcohol	0.3 per cent.	28
Normal atmospheric pressure		100
CO_2-free atmosphere		60
Increased CO_2 pressure		300
Hydrogen atmosphere		50
Increased atmospheric pressure (5-10 per cent.)		130
Reduced atmospheric pressure (10 per cent.)		100

It is seen from the above data that the nitrates have a depressing effect upon the respiration of the organism while the sulfate does not. 0.025 molar concentration of the nitrate is sufficient to reduce respiration to 27-29 per cent.; 0.05 molar only to about 10-20 per cent., while still higher concentrations practically depress respiration. The action of nitrates is not antagonized by the presence of ammonium sulfate, since 0.025 molar nitrate depresses respiration in the presence of an equimolar concentration of ammonium sulfate to the same extent as in its absence.

Peptone proves to be another substance injuring respiration even in comparatively small concentrations. The injurious action of nitrates and peptone can also be readily demonstrated by adding these substances to the culture media upon which the organism is grown. The amino acids and acid amides have some depressing effect, but not to such an extent as peptone.

Cyanides repress respiration completely, even in concentrations of 0.0004 molar. Dextrose has no depressing effect on respiration, even a 1 per cent. concentration tends to favor it.

The influence of the gas pressure is of particular interest, particularly that of carbon dioxide. When the flasks, in which respiration was taking place, were covered with air tight bell jars, it was found that a CO_2-free atmosphere reduces respiration to 60 per cent., while an atmosphere rich in CO_2 (which was allowed to bubble from a gas tank into the bell jar) in-

creased respiration 300 per cent. An hydrogen atmosphere (no precautions were taken to free the gas from traces of oxygen and CO_2) reduced respiration to 50 per cent. Reducing the atmospheric pressure 5-10 per cent. has no appreciable effect on respiration, while increasing the pressure 10 per cent. has a stimulating effect.

The ratio between the sulfur oxidized and carbon assimilated by the culture is about 32, this ratio varying greatly by changing the conditions of growth and by adding various depressive substances. The presence of nitrates, for example, greatly increases the ratio. When thiosulfate is used as a source of energy the ratio is about 65. Of the total amount of energy made available only about 6.5 per cent. is utilized by the organism. The amount of energy utilized by the nitrite and nitrate bacteria is about 5 per cent. These quantities in comparison with the low utilization of energy by higher plants point to the greater efficiency of the autotrophic bacteria.

4 (1964)

A study of light waves in relation to their protective action in rickets

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In a communication presented a year ago we showed that rickets can be prevented in rats by daily exposures to direct sunlight for about fifteen minutes[1]. A similar result was reported at the same time by others[2]. When rats were placed in a box having flint glass windows it was found that the sun's rays, in traversing the glass, had lost their protective power. In a later communication it was shown that the pigment of the skin also hinders the action of the effective rays, that black rats require more radiation than do white rats[3].

[1] Hess, A. F., Unger, L. J., Pappenheimer, A. M., Proc. Soc. Exp. Biol. and Med., 1921, xix, 8.

[2] Shipley, P. G., Park, E. A., Powers, G. F., McCollum, E. V., Proc. Soc. Exp. Biol. and Med., 1921, xix, 43.

[3] Hess, A. F., Unger, L. J., Pappenheimer, A. M., Proc. Soc. Exp. Biol. and Med., 1922, xix, 238.

These experiments have been extended to ascertain more nearly the wave lengths which exert the protective action. For this purpose glass filters have been used. These filters are manufactured by the Corning Glass Works and have been tested by this establishment and by the United States Bureau of Standards, both in regard to the wave lengths which they transmit, and their percentage of transmission[4]. In this work white rats have been used which were fed the standard rickets-producing dietary (No. 84). Previous experiments have shown that rats on this dietary can be protected against rickets by daily irradiation for two minutes or less, by the mercury vapor quartz lamp at a distance of three feet with a voltage of 76 (a unit dose). The method of procedure was to interpose the various filters and to ascertain to what extent they altered the protective action of the light at exposures of varying intensity. The animals were radiographed after an interval of about 21 days, and killed after 28 days. The interpretation of rickets was based on a microscopic examination of the epiphyses.

The accompanying chart illustrates a series of experiments with various filters. The first (G38H) did not transmit waves shorter than 475$\mu\mu$. It will be noted that protection was not afforded, although irradiation was carried out for 60 minutes at a distance of only 9 inches (480 units; the protective dose being about 2 minutes at 36 inches). Window glass which transmitted rays as short as 334$\mu\mu$ also obstructed the protective rays. Filter G586A allowed the passage of a very small percentage of rays as short as 313$\mu\mu$, and a very small percentage of 302$\mu\mu$ rays. With this filter protection was afforded when long exposures were resorted to. Pyrex glass which transmits a much larger percentage of rays of 313$\mu\mu$, as well as shorter rays, interfered but slightly with the action of the mercury vapor lamp.

From these experiments we may conclude that rays as long as 334$\mu\mu$ have little or no protective action in rickets and that the effective rays begin in the neighborhood of 310$\mu\mu$. The degrees of transmission of the various wave lengths will be discussed in detail in the full report of this work.

The experiment with filter G86B is of special interest. This filter transmits short rays of about the same wave lengths and

4 Technologic Papers of the Bureau of Standards, No. 119 and 148, also Scientific Paper No. 325.

intensity as does G586A. It will be noted, however, that when the latter was used rickets did not develop with long exposures, whereas when G86B was interposed marked rickets developed, even with exposures of 60 minutes at a distance of 9 inches. The chief difference between these filters is that G86B, which is a nearly neutral filter, allows far more of the longer visible rays to pass than does G586A, which is a purple filter. Further experiments are in progress to ascertain whether the interference of visible rays can account for these divergent results.

Filters of various clothing material were also employed. It was found that woolen as well as cotton goods interfered with the activity of the light in proportion to their thickness, but did not prevent protection if the dosage of irradiation was made adequate. Black cotton material filtered out the protective rays to an extent greater than white material of the same weave.

5 (1965)

The mechanism of bacteriostasis.

By JOHN W. CHURCHMAN

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The effect of bactericidal agents is often tested by adding these substances to the media on which the organisms are planted; and the assumption is usually made that if the substances, when present in the media, exhibit a selective hostility to bacteria they will exhibit a hostility—selective in the same sense—when added directly to the organisms themselves. This assumption is usually justified by the facts; no single exception to such a parallelism has been met with in the large number of experiments made with gentian violet and allied tri-phenylmethanes. We are in the habit therefore of reasoning from experiments which test bacteriostasis to conclusions as to bactericidal value, at least so far as selective features are concerned.

Proof will here be presented to show that this sort of reasoning is not always justified by the facts. Suppose for example that we plant B. *prodigiosus* and B. *megatherium* on acid fuchsin agar and find that B. *prodigiosus* grows well and B. *megatherium*

not at all. We would then certainly expect that if these two organisms were exposed to acid fuchsin and planted on plain agar the latter would be killed and the former unaffected. As a matter of fact the opposite occurs. A similar result is obtained if the experiment be done with the flavines instead of with acid fuchsin.

If we describe the bacteriostasis which a substance exhibits when bacteria are exposed to it (before being planted on plain agar) as *intrinsic* bacteriostasis, and that which a substance exhibits when it is present in the media on which the unstained bacteria are planted, as *extrinsic* bacteriostasis: then we may say that the intrinsic selective bacteriostatic features of a substance so far from necessarily running parallel with its extrinsic selective features may run directly counter to them. There seems little doubt that these facts explain in part some of the discrepancies between laboratory experiments and clinical results.

Since certain dyes, like gentian violet, are highly bacteriostatic for some organisms which they penetrate little if at all (e.g., subtilis spores), and are almost without bacteriostatic effect on other organisms which they have penetrated thoroughly (e.g., *b. coli*) it is certain that selective bacteriostasis is not entirely dependent on selective penetration. There is considerable evidence for the assumption that selective bacteriostasis depends on H-ion concentration at the surface of the bacteria.

FILTRATION OF RAYS (MERCURY VAPOR QUARTZ LAMP)

Rat No.	Filter	Exposure		Lower Limit of Spectra of Filters	Rickets	
		Time (min.)	Distance Inches		Radiograph	Microscopic Examination
949	G 38 H	10	12	475 $\mu\mu$	moderate	marked
950	(4.0 mm.)				moderate	marked
951					moderate	marked
1063	G 38 H	30	9	475 $\mu\mu$	moderate	moderate
1064	(4.0 mm.)				moderate	moderate
1065					moderate	slight
1066					moderate	moderate
1204	G 38 H	60	9	475 $\mu\mu$	moderate	moderate
1205	(4.0 mm.)				moderate	moderate
1206					moderate	slight
1207					moderate	slight

1302	Window	15	36	334 $\mu\mu$	marked	marked
1303	Glass				marked	marked
1304	(2.6 mm.)				marked	marked
1305					marked	marked
1493	Window	30	9	334 $\mu\mu$	marked	marked
1494	Glass				marked	marked (slight calcification)
	(2.6 mm.)					
1488		60	9	334 $\mu\mu$	marked	marked
1489					moderate	marked
1490					moderate	marked
1491					marked	marked
952	G 586 A	10	12	313 (302) $\mu\mu$	moderate	extreme
953	(4.32 mm.)				moderate	marked
954					moderate	extreme
1067	G 586 A	30	9	313 (302) $\mu\mu$	very slight	minimal
1068	(4.32 mm.)				slight	minimal
1069					slight	minimal
1070					neg. (?)	almost neg.
1200	G 586 A	60	9	313 (302) $\mu\mu$	neg. (?)	negative
1201	(4.32 mm.)				neg. (?)	slight
1202					neg. (?)	negative
1203					neg. (?)	negative
1330	Pyrex	15	18	289 (280?) $\mu\mu$	negative	No. R.
1331	(0.8 mm.)				negative	No. R.
1332					negative	No. R.
1333					negative	No. R.
1516	Pyrex	6	18	289 (280?) $\mu\mu$	negative	No. R. osteoporosis)
	(0.8 mm.)					
1517					negative	
1518					negative	No. R. (osteoporosis)
1519					negative	No. R.
1512	Pyrex	3	18	289 (280?) $\mu\mu$	slight	slight
1513	(0.8 mm.)				negative	slight
1514					slight (?)	slight
1515					slight (?)	slight
1334	G 86 B	15	18	313 (302) $\mu\mu$	marked	marked
1335	(4.1 mm.)				marked	marked
1336					marked	marked
1337					marked	marked
1500	G 86 B	30	9	313 (302) $\mu\mu$	moderate	marked
1501	(4.1 mm.)				moderate	marked
1502					moderate	marked
1503					moderate	marked
1496	G 86 B	60	9	313 (302) $\mu\mu$	moderate	marked (slight calcification)
	(4.1 mm.)					
1497					marked	marked
1498					R (?)	marked
1499					moderate	marked

6 (1966)

Bacteriostasis with mixed dyes.

By JOHN W. CHURCHMAN

[*From the Department of Hygiene, Cornell University Medical College, New York City*]

Since the time of Ehrlich, chemotherapy has been largely concerned with efforts to fortify the weaknesses of bactericidal and paracitocidal substances by chemical manipulation of the molecule. It has been recently found, however, that the end in view may, in some cases, be easily achieved by the use of simple mixtures of certain dyes which have been previously shown to possess opposite selective bacterio-static properties. The fact was reported to this society last year that acid fuchsin possesses a selective bacteriostatic power which is in many important respects the opposite of the selective power possessed by gentian violet. Acid fuchsin and gentian violet will not, however, mix. I have recently shown that neutral acriflavine possesses, like acid fuchsin, a reverse selective bacteriostatic power; and acriflavine and gentian violet *will* mix. Moreover the resultant substance acts as a mixture. It is thus possible to fortify the weakness of each of these two dyes with the strength of the other. If a mixed bacterial emulsion containing *B. pyocyaneus* and *B. anthracis* be exposed to gentian violet and streaked on plain agar a pure culture of *B. pyocyaneus* will result. If the same mixed emulsion be exposed to neutral acriflavine a pure culture of *B. anthracis* results. If the emulsion be exposed to a mixture of the two dyes neither organism grows. The principle of supplementary selective bacteriostasis by means of mixtures is therefore established; and the possibility of mixing at least certain dyes, whose selective bacteriostatic powers are opposed, without the formation of a new substance is demonstrated.

7 (1967)

The acid base ratio of the diet in rickets production.

By T. F. ZUCKER, WM. C. JOHNSON and MARION BARNETT

[*From the Laboratory of the Department of Pathology, College of Physicians and Surgeons, Columbia University, New York City*]

The results of the work on experimental rickets in rats have shown that rickets can be produced by diets which are poor in phosphorus and rich in calcium or diets which are poor in calcium and rich in phosphorus. A condition produced in that manner can be prevented or restored to normal by exposing the animals to a sufficient amount of light of certain wave lengths or by administering cod liver oil which contains a substance that affects the calcium and phosphorus metabolism. There is no evidence at present that this substance regularly occurs in natural foodstuffs, and, therefore, there seems no valid reason to put it into the class of vitamins. Until it has been shown that the curative agent in cod liver oil is a component of normal foods, we cannot assume that rickets is due to a vitamin deficiency. This, then, leaves us, in spite of the accumulated data on experimental rickets in animals, without any definite information as to the physiological changes which lead to rickets in children since children will develop rickets on diets well balanced with regard to calcium and phosphorus. It is important, therefore, to learn more concerning other factors which influence rickets-production, in order to gain an understanding of the possible causes of human rickets when it is produced under conditions where dietary factors cannot be held responsible. We believe that we have obtained evidence of a factor not taken into consideration in the recent work on experimental rickets. Ernst Schloss[1] in one of his monographic papers on rickets has called attention to the fact that contrary to the hypothesis held at one time, according to which rickets is due to an acidosis, it is found that rickets seems to develop more frequently under conditions rather the opposite of an acidosis. There are also certain indications that during cure of rickets a relative acidosis develops. Schloss, however, had no very def-

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[1] Schloss, Ernst, *Ergebn. inn. Med. u. Kinderh.*, 1917, xv, 95.

inite data on which to base this assumption. Furthermore, Schabad[2] has shown that in active rickets the distribution of calcium and phosphorus between urine and feces is such as we now know will occur with insufficient acidity of intestinal contents. We believe that in some of our rat experiments we have supplied evidence pointing in the direction of Schloss's hypothesis. On a diet consisting of flour, casein, calcium lactate, sodium chloride, and a trace of ferric citrate, rats will regularly develop a rather marked rickets as shown by X-ray and microscopic sections of ribs. This diet, like practically all diets used in rickets production, contains a large excess of base-ion over acid-ion, the lactic acid of the calcium lactate being a weak acid, while calcium is a strong base. When we substitute, in this diet, calcium chloride for calcium lactate in equivalent quantities, we markedly increase the acidity. Such a diet, when fed to rats, resulted in bone growth so nearly normal that it could not be called more than minimal rickets, while the control rats getting calcium lactate developed a very marked rickets. In another set of experiments, we made the rickets producing diet mentioned above more acid by addition of 2 per cent. of ammonium chloride. The rats receiving ammonium chloride developed no rickets, while those on the control diet showed, as always, perfectly definite rickets. We think, therefore, that without modifying any of the factors discussed heretofore, we can change a rachitic into a non-rachitic diet.

The reverse of the above experiments was obtained on certain diets made up of flour and egg albumen together with a suitable salt mixture. The control rats were given an egg albumen-flour diet to which enough potassium phosphate had been added to prevent development of rickets. To this diet was added 2 per cent. of sodium carbonate making the diet extremely alkaline. On the latter diet rats developed a very marked rickets. Thus we have changed a non-rachitic diet into a rachitic diet without changing the calcium or phosphorus intake in the food or any of the other known factors which affect rickets. The growth curves of these rats were all approximately the same, thus ruling out the question of total food intake.

If we consider that the digestive tract is capable of a good deal

2 Schabad, *Ztschr, f. klin. Med.*, 1909, lxviii, 94.

of variation in hydrogen ion concentration through the varying growth, we will see what the significance of these experiments may be. In the first place, it is well known that the hydrogen ion secretion of digestive juices and also possibly through bacterial concentration of the gastric juice of infants on a milk diet is only a small fraction of that of older children and adults on mixed diet. Therefore, it is quite possible that through a still further reduction of acid in the digestive tract a significant lack of absorption of calcium and phosphorus may take place. We reported experiments in these Proceedings[3] some time ago on the effect of varying intake of acid and base on the partition of calcium and phosphorus between urine and feces, and interpreted the effect of greater urinary excretion under a more acid regime as a greater absorption from the intestine. Data supplied by Scheer[4] in the meantime further substantiate this, and show that in children there is concomitant with a greater absorption under the influence of acid, also a greater retention of calcium and phosphorus.

We come to the conclusion, therefore, that on a diet which from the point of view of balance between calcium and phosphorus should not lead to rickets, it may be possible that with a change in the intestinal tract in the sense of a lessening acidity, rickets may be produced. Here we have, then, a factor which resides in the individual and not in the diet, and may lead to at least a partial explanation of the occurrence of rickets in infants on a diet which is perfect from a dietetic point of view.

In the course of this winter we are planning to investigate the application of this hypothesis to infantile rickets.

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[3] Zucker, T. F., PROC. SOC. EXP. BIOL. AND MED., 1921, xviii, 272.

[4] Scheer, *Jahrb. f. Kinderh.*, 1922, xcvii.

8 (1968)

Observations of fluctuations of virulence of B. influenzæ.

By FREDERIC PARKER, JR. and JULIA T. PARKER

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Since the relationship of the Pfeiffer bacillus to influenza is still a matter of dispute, any observation having a bearing on its epidemiological properties should have some value. This is particularly true since the epidemiology of this disease presents several features unexplainable at the present time, such as the difference in morbidity and mortality between the first and second waves, the success of isolating the organism in the disease at one time and the failure at other times, the negative experiments in human inoculations, etc.

The work reported here may be divided into three parts:

    I. Results obtained working with a virulent meningeal strain.

    II. Results obtained working with the same strain when less virulent.

    III. Results with other less virulent strains.

I. The experiments in this part were done with a strain of *B. influenzae* isolated from the spinal fluid of a case of influenzal meningitis at the Nursery and Childs' Hospital on October 5th, 1921. This strain was kept in whole defibrinated rabbit's blood without transplantation until December 21st, about 10 weeks after isolation, when this work was started. When the organism was first received on artificial media, it was extremely pleomorphic, showing many long forms and forms of considerable size. It is interesting to note that later in exudates from the animal body, the organisms were extremely small and coccoid in shape; in fact, they looked so small that we attempted to filter them through a Berkfeldt N but with negative results.

We inoculated rabbits in the following ways with the results given below:

1. *Intratracheal.* Rabbits were injected at first with chocolate broth cultures, then, in the succeeding experiments, with varying amounts of exudates of animals dead from inocula-

tions. As a rule, the animals died three or four days after inoculation, about 50 per cent. of them showing a septicæmia of *B. influenzae.* In this series, all the animals, eight in number, so inoculated, died, but one. At autopsy the usual picture was marked fibrinous pleuresy with a pleural exudate of cloudy fluid; the lungs in two animals showed pneumonia, in the remainder they were apparently negative; in five cases there was a fibrinous exudate covering the trachea and extending down into the sup. mediastinum. One animal showed a serofibrinous peritonitis. All other organs were apparently negative. Microscopically the lungs from five animals were examined; two with pneumonia in gross, and three apparently negative. However, of these three, two showed small focal areas of pneumonia microscopically. One case also showed necrosis of the heart muscle fibres. Smears of the pleural exudates showed very numerous organisms and but a few leucocytes. The organisms were extremely small and coccoid in shape. Cultures were positive from the pleural exudate and neck exudate, and in about 70 per cent. from the heart's blood. One animal was injected by mistake in the neck tissues instead of into the trachea and died overnight with a septicæmia. This would tend to settle the question as to whether we were not causing the above lesions by missing the trachea and injecting into the neck, for death in these animals where we felt sure we were in the trachea did not occur for three or four days, never overnight as in this animal. In all instances the animal was anesthesized and the trachea exposed by operation.

2. *Intracerebral.* Three animals were inoculted with the following doses: 0.005 c.c. of the pleural exudate of a rabbit killed by intracheal inoculation, 0.001 c.c. of the peritoneal exudate of a rabbit inoculated intraperitoneally, and 0.1 c.c. of a 20 hour chocolate broth culture. Blood cultures on two of the rabbits taken $3\frac{1}{2}$ hours and five hours, respectively, after inoculation were positive; the third animal was not cultured before death. At autopsy cultures of the heart's blood in all three were positive. Pathologically, nothing abnormal was found with the exception of hæmorrhage into the thymus in one animal.

3. *By stomach tube.* Five animals were inoculated in the stomach by means of a stomach tube. Of these, two died, one receiving 15 c.c. of 20 per cent. chocolate broth culture, and the

other 9 c.c. of 20 per cent. chocolate broth culture plus 1 c.c. of pleural exudate of rabbit dead of intratracheal inoculation. A blood culture was taken on one of the rabbits 6 hours after inoculation and was positive. At autopsy cultures of the heart's blood of both were positive. Of the three that did not die, one received only 2 c.c. of broth culture, one received 20 c.c. of broth culture inoculated with the organism after a week's growth on chocolate agar, and the third, who received the same dose as one that died, had previously received two small inoculations into the stomach. The two animals that died showed increased respiration and high temperatures almost immediately. The mechanism of death in these cases has not been worked out and at present is not clear.

II. After carrying out the above experiments, we next worked with other strains. About three weeks later, we returned to our meningeal strain "M," but, as the strain we had passed through the animals in our previous experiments had died out, we had to go back to the original strain in rabbit's blood, which then had been out of the body 18 weeks, instead of 10 weeks, as in our first series of experiments. To our surprise, we could not repeat our former experiments as the virulence of the organism had diminished to such a degree. Our results were as follows:

1. *Intratracheal.* Three rabbits were injected with 3.5 c.c., 4 c.c., and 4 c.c. of 20 per cent. growth in chocolate broth, respectively, and one with 2 c.c. of the peritoneal exudate of a guinea pig dead from an inoculation. None of these animals died, or even were sick.

2. *Intracerebral.* One animal was inoculated with 0.1 c.c. of heavy emulsion of a culture on a chocolate agar slant; it died in 48 hours; at autopsy the heart's blood and brain was positive for *B. influenzae,* but blood cultures 6 hours after inoculation and 24 hours later were negative. The other animal received 0.1 c.c. of an emulsion of the brain of the first rabbit; it did not die, nor was it sick.

3. *By stomach tube.* One animal was given 20 c.c. of a 20 hour growth inoculated with the blood by one rabbit dead with a septicæmia following an intraperitoneal injection. It never showed any reaction. The other animal received 18 c.c. of a 20 hour chocolate broth culture plus 2 c.c. of the peritoneal exudate of a guinea pig dead from an intraperitoneal inoculation.

III.   The three strains worked with in this group were "R," a meningeal strain from the spinal fluid of a case of influenzal meningitis; "S," isolated from the sputum of an acute case of influenza; "J," isolated from the pus of a case of chronic infection of the antrum, and "Z," from the sputum of a case of acute coryza.

A.   The following experiments were done with "R":

1. *Intracerebral.*   One rabbit was injected with 0.1 c.c. of spinal fluid from a case of influenzal meningitis shortly after removal from the patient. Blood culture on the animal next day was negative. He never was sick, and at the end of three days was used in another experiment.

Another rabbit was injected with 0.1 c.c. of the peritoneal exudate of rabbit killed by intraperitoneal injection with a chocolate broth culture of this strain. Blood cultures 24 hours later showed approximately 500-1000 colonies in a drop of blood. Blood 48 hours after injection showed 10 colonies to a drop of blood. Blood culture 72 hours and 96 hours after injection were negative. The animal died 10 days after inoculation; at autopsy, the brain showed a yellowish caseous area at the inoculation site, 0.2 x 0.2 x 0.1 cm.; cultures from this lesion and the heart's blood were negative.

B.   The following experiments were done with strain "S":

1.   *Intratracheal.*   One rabbit received 2.5 c.c. of the patient's sputum; it never was sick and blood cultures were uniformly negative.   The animal was killed with ether 9 days after; autopsy findings and cultures from the lungs and heart's blood were negative.

2.   *Intracerebral.*   Four rabbits were injected.

(a)   Received first 0.75 c.c. of filtrate of "S" on chocolate broth intravenously with symptoms typical of poisoning with filtrates of cultures of *B. influenza* (1); while still sick, one and a half hours later he received intracerebrally 0.1 c.c. of the centrifuged sediment of a 20 hour chocolate broth culture. Blood culture 6 hours later and 24 hours later was negative. It died the night of the second day. At autopsy, the left lateral ventricle showed on its inferior surface a few yellowish spots and injection of its upper surface; the rest of the organs were negative; smears and cultures of the fluid in the ventricles were positive while the heart's blood were negative.

(b)   Was inoculated with 0.1 c.c. of an emulsion of the brain of (a).   24 hours later, it was sick and unsteady on its feet; blood culture was negative.   48 hours later nervous symptoms were very marked, consisting of ataxia and increased irritability; it was so sick in the afternoon of this day that we killed it with ether.   At autopsy cultures from the brain and heart's blood were negative.

(c)   Received 0.1 c.c. of heavy emulsion of organisms from a chocolate agar slant, inoculated from organism recovered from brain of (a).   Blood cultures were positive 6 hours, 24 and 48 hours after inoculation, but were negative at 72 and 96 hours.   The animal was sick 24 hours later, showing ataxia and dyspnea; it became progressively worse, developing a stiff neck and finally was killed with ether four days after inoculation as it was so sick.   At autopsy, the brain at the site of inoculation showed an area of yellowish friable material 0.3 cm. in diameter, extending 0.2 cm. into the substance of the cortex.   Cultures from the brain were positive, but the heart's blood was negative.

(d)   Inoculated with 0.1 c.c. of emulsion of brain of (b) which was shown to be sterile.   At the end of three days, it developed diarrhea and looked sick; two days after this, it died. At autopsy the brain at the inoculation site showed an area of yellowish friable material 0.2 x 0.1 cm.   Smears and cultures of this area, the ventricular fluid and the heart's blood were negative.

3.   *By stomach tube.*   One animal was given 20 c.c. of a 20 hour growth in chocolate broth.   It was never sick.   Blood culture seven hours after inoculation was negative.

C.   Experiments with strain "J":

1.   *Intracerebral.*   One animal was given 0.1 c.c. of a 20 hour chocolate broth culture.   Blood culture was negative 24 hours later.   It never was sick.

2.   *By stomach tube.*   One rabbit received 27.5 c.c. of a 20 hour chocolate broth culture and never was sick.

D.   Experiments with strain "Z":

1.   *Intracerebral.*   One rabbit received 0.1 c.c. of a 20 hour chocolate broth culture.   He developed no symptoms so that 3 days later was given 2 c.c. of a similar culture intraperitoneally; following this, blood cultures 5 hours and 24 hours later were

negative. As he was not very sick after the second inoculation, he was again injected intraperitoneally with 2 c.c. of a chocolate broth culture in the morning, and 4 c.c. of the same culture in the afternoon. The following day he was perfectly well. 15 days after his brain inoculation, he developed paralysis of the hind legs, his respiration was labored and in general he was so sick that we etherized him to death. At autopsy, the brain alone showed any pathological changes; at the inoculation site there was a yellowish caseous area 0.2 x 0.2 x 0.1 cm. Smears and cultures of this area and the heart's blood were negative.

A second rabbit was given 0.2 c.c. of an emulsion of the brain of the preceding rabbit. He never developed any nervous symptoms, but a week later developed diarrhea. He did not die.

DISCUSSION.

On comparing the results of I and II, the striking difference in virulence will be noted. In II we were unable to kill or even make sick with intratracheal or stomach inoculations, and while we killed one animal with an intracerebral inoculation, in this case we used a very large dose, and the animal did not develop a septicæmia a few hours later as did the animals in I. On the whole, the results in II closely resembled those obtained with the strains we worked with in III.

There is one interesting feature in the less virulent group which deserves attention, namely, that in two animals that developed a septicæmia 24 hours and 58 hours after inoculation, this septicæmia then cleared up and yet the animals died, in one instance at least, all cultures being negative at autopsy. Three rabbits inoculated intracerebrally, one rabbit inoculated intrapleurally and a mouse injected with sputum containing numerous B. influenza died with negative cultures at autopsy. This is an important point, especially in connection with human cases, for it brings up the possibility that an individual may die of an infection with B. influenzæ and yet all cultures at autopsy fail to show evidence of this organism. The most probable explanation of this seems to be that at first when the organisms are growing they elaborate a poison causing a fatal injury, and then later the defenses of the body or some other factor kills off the organisms, but too late to prevent death as the injury has been done.

By our results in I and II, we have shown that apparently the length of time out of the body has a definite influence on the virulence of the organisms. That the virulence may diminish in the course of the disease is suggested by a single observation: A mouse was injected with the sputum of a case of influenza on the first day of the disease; it died 6 hours later with positive cultures of *B. influenzæ* in the peritoneal exudate and heart's blood; on the second day of disease, some more sputum, smears of which showed numerous *B. influenzæ*, from this patient was injected into a mouse; this mouse, however, did not die for 48 hours, and at autopsy cultures from the heart's blood and peritoneum were negative.

Conclusions.

1.   The virulence of *B. influenza* is very variable and this may have some bearing on the epidemiology of the disease influenza in man.

2.   A few observations are present that may throw some light on the results of bacteriological studies of this disease.

9 (1969)

The formol titration of bacteriological media.

By J. HOWARD BROWN

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The formol titration devised by Malfatti[1] (1908), Sörensen (1907[2], 1908[3]), and by Henriques and Sörensen[4] (1909) for the titration of the ammonia and amino acids of urine has been more or less modified by bacteriologists for the titration of media and cultures. Ammonium chloride reacts with formaldehyde to produce hexamethylentetramine and hydrochloric acid. Amino acids and polypeptides react with formaldehyde to produce acid methylene derivatives which are stronger acids than the amino acids

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[1] Malfatti, H., *Z. f. anal. Chem.*, 1908, xlvii, 273.

[2] Sörensen, S. P. L., *Comptes-rendus des Travaux du Lab. de Carlsberg*, 1907, vii, I.

[3] Sörensen, S. P. L., *Biochem. Zeitschr*, 1908, vii, 45.

[4] Henriques, V., and Sörensen, S. P. L., *Z. f. physiol. Chem.*, 1909, lxiii, 27.

from which they are derived. The increase in acidity may be titrated against a standard alkali solution and serve as a measure of the amino acids and ammonia present in the sample.

Sörensen (1907, 1908) pointed out that the reaction of amino acids with formaldehyde is a reversible one. A considerable excess of formaldehyde is required to effect complete conversion of the amino acids into their methylene derivatives, and, since water is a product of the reaction, the presence of too much water serves to throw the reaction back in the opposite direction. Sörensen found it necessary to add as much as 10 c.c. of formalin (40 per cent. formaldehyde) to 20 c.c. of amino acid solution.

The amino acids are ampholytes. If a pure monocarboxylic amino acid is dissolved in distilled water the solution will be found to have a hydrogen ion concentration at or near the isoelectric point of the amino acid, a fact also noted by Eckweiler, Noyes and Falk[5] (1921). If it is titrated with alkali and the titration curve plotted with amounts of alkali added as abscissae and hydrogen ion exponents as ordinates the curve will be seen to drop almost vertically toward the alkaline side as the first drops of alkali are added. This represents a portion of the "isoelectric zone" (Michaelis[6], 1914, p. 40). As more alkali is added the curve assumes a more nearly horizontal position and if the curve is continued it again assumes a nearly vertical position as the point of complete neutralization of the amino acid is approached (at about P_H 11.3 in the case of glycine). This latter vertical portion of the curve may be called the "zone of neutralization." When formalin is added the amino acid solution becomes more acid not because the carboxyl groups are increased but because the amino groups are destroyed. The titratable acidity of the acid methylene derivative is the same as that of the amino acid but the point of neutralization of the methylene derivative lies at a higher hydrogen ion concentration (at about P_H 8.0 in the case of methylenglycine) than does that of the amino acid. For the formol titration of an amino acid the solution should be reduced to a hydrogen ion concentration within its isoelectric zone and after the addition of formalin the titration

[5] Eckweiler, H., Noyes, H. M., and Falk, K. C., *J. Gen. Physiol.*, 1921; iii, 291.

[6] Michaelis, L., *Die Wasserstoffionenkonzentration*, Berlin, 1914.

should end at a hydrogen ion concentration within the zone of neutralization of the methylene derivative. To determine the location of these zones we have plotted the titration curves of a number of representative amino acids and ammonium salts before and after the addition of formalin[7]. The following pure substances were titrated: glycine, alanine, phenylalanine, tryosine, asparagine, aspartic acid, glutamic acid, ammonium chloride, ammonium lactate, ammonium phosphate, ammonium carbonate. Excluding ammonium phosphate and ammonium carbonate which present a special problem it is found that the isoelectric zone of each of these substances extends beyond P_H 6.0 and as far as P_H 7.0, some as far as P_H 8.0[8]. After the addition of formalin the zone of neutralization for each substance was found to begin on the acid side at about the hydrogen ion concentration here listed; glycine P_H 6.8, alanine P_H 8.0, phenylalanine P_H 7.6, tryosine P_H 7.6, asparagine P_H 6.0, aspartic acid P_H 8.0, glutamic acid P_H 8.0, ammonium chloride P_H 6.0, ammonium lactate P_H 6.0, ammonium phosphate P_H 8.0, ammonium carbonate P_H 7.0. For the formol titration of most amino acids or of mixtures of amino acids, therefore, their solutions should be reduced to a hydrogen ion concentration of not less than P_H 7.0 and after the addition of formalin the titration should end at a hydrogen ion concentration not greater than P_H 8.3[9]: Theoretically P_H 8.4 is a better end point than P_H 8.0 but we have found the buffer effect of the formalin so great at P_H 8.4 that the end point of the titration judged colorimetrically is very poor, and have obtained more accurate results at P_H 8.0.

Sörensen recognized phosphates and carbonates as disturbing elements in the formol titration of urine. In fact any substance which exerts a buffer effect between P_H 7.0 and P_H 8.0 is a serious source of error. To overcome this difficulty Henriques[10] (1909) and Henriques and Sörensen (1909) described methods for the

[7] A correction was made for the buffer effect of the formalin itself.

[8] Of the substances titrated, tyrosine showed the greatest degree of dissociation at P_H 7.0 and was about 3.8 per cent. dissociated at this hydrogen ion concentration. Glycine, leucine and alanine are less than 2 per cent. dissociated at P_H 8.0.

[9] Henriques and Sörensen (1909, 1910) recommend titration from P_H 6.8 to about P_H 8.4 or beyond. Northrop (1921) started his titration at P_H 7.0.

[10] Henriques, V., *Z. f. physiol. Chem.*, 1909, lx. 1.

removal of phosphates and carbonates by precipitation with barium and filtration. These methods may be adopted by the bacteriologist, but are time-consuming. If many cultures are to be titrated the element of time is very important. Of almost equal importance to the bacteriologist is the ability to make determinations with small samples of material. Bacterial cultures present a special problem in some respects. Their color and turbidity are not easily matched by artificial controls. They may contain other buffer substances than phosphates and carbonates. We have tried to reduce the formol titration of bacterial cultures to its simplest terms. Color and turbidity may be overcome and much greater accuracy in judging a colorimetric end point may be attained by the use of a comparator block and Clark and Lubs' indicator solutions. The error due to the presence of buffer substances may be eliminated by starting and ending the titration at the same hydrogen ion concentration. If the isoelectric zone of an amino acid or ammonium salt overlaps the zone of neutralization of its methylene derivative this may be done with perfect results. This is the case with glycine, alanine, asparagine, ammonium chloride, ammonium lactate and probably with others not studied. In the case of the other amino acids titrated there is no actual overlapping of the zones, but there are certain hydrogen ion concentrations at which the zones approach each other closely. The overlapping of the zones or their points of nearest approach are not at the same hydrogen ion concentration for all the substances titrated. It was determined empirically in the case of standard bouillon and bouillon cultures of various bacteria that maximum formol titrations were obtained when the reaction of the sample was reduced to P_H 8.0, formalin added, and the mixture immediately titrated back to P_H 8.0. The results so obtained were reasonably close (within about 6 per cent.) to those obtained by titration from P_H 7.0 to P_H 8.0 after removal of phosphates and carbonates by precipitation with barium and filtration. The details of the method recommended are in press.

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[11] Henriques, V., and Sörensen, S. P. L., *Z. f. physiol. Chem.*, 1910, lxiv, 120.

10 (1970)

The relation of the position of the normal and the enlarged heart to the electrocardiogram.

By ALFRED E. COHN and MILTON J. RAISBECK

[*From the Hospital of the Rockefeller Institute for Medical Research, New York, N. Y.*]

It is well known that when the heart enlarges, it gives rise to alterations in the electrocardiogram; the curve takes on one form when the left ventricle is hypertrophic as in aortic insufficiency; it takes on an opposite form when the right ventricle is principally involved, as in mitral stenosis. These electrical signs have been useful in the clinic, but they have on occasion been misleading, for we have obtained curves resembling those found in enlarged hearts, in young soldiers and in civilians who, we were quite certain, were not the subjects of disease. It occurred to us that the position of the heart in the chest might be an important contributing factor in producing the abnormal electrocardiograms. It is well known that changes in the curves are possible as the result of respiratory movement; of the posture of the body. An exaggerated instance occurs in dextrocardia, as the result of which the electrocardiogram assumes the appearance of a mirrored image of the usual curve. Having these experiences to guide us we planned to ascertain in a systematic manner precisely what was the effect of the position of the heart in the chest. Obviously we could not rotate the heart in the chest, but we could rotate the chest about the heart Our method was as follows: Instead of taking the usual limb leads, we placed electrodes at the apices of the largest equilateral triangle which we could apply to the chest, the base of the triangle stretching between the shoulders, the apex below the sternum. We assured ourselves that the curves taken from these leads resembled closly those from the limb leads. We proceeded then to rotate this triangle through successive arcs of 40°, taking curves at each new position from those points of the chest wall to which the apices of the triangle pointed. We photographed the three leads simultaneously, using three galvanometers. By this means, we did in effect rotate the chest about the heart. In normal in-

dividuals, by small changes in the position of the triangle of leads, resulting in a change of the anatomical angle of the heart of 40° or less, we obtained curves which actually resembled curves found in disease and resembled closely those often thought to be abnormal found in the young soldiers and civilians already mentioned. These were curves like those seen when the left side of the heart is large; in other positions, though anatomically quite improbable, we obtained curves resembling enlargement of the right side of the heart.

In patients whose hearts were known to be diseased and whose curves were characteristic of these conditions we applied the same technique. We found that at some position in the course of rotating the leads, curves were obtained which approached the appearance of normal.

That curves of approximately normal appearance can be obtained is of theoretical interest. But that in obviously normal persons, yielding abnormal curves, normal curves can be obtained by a slight alteration of the position of the heart in the chest is a matter of genuine practical importance. It does not simplify the interpretation of electrocardiograms, but for the moment it does something quite as important; it shows how the normal individual may be saved from being considered the subject of chronic heart disease because of an abnormality in his curve. This danger is not theoretical.

We have not by these experiments solved the problem of the relation of hypertrophy of the heart to patent abnormality in the electrocardiogram, but we have shown systematically and clearly that, especially in doubtful cases, the position of the normal heart in the chest must be taken into account as a factor in the production of abnormal curves as well as the position of the abnormal heart in the chest in the production of normal ones.

11 (1971)

A phyto-pharmacological study of some isomers.

By DAVID I. MACHT

[*From the Pharmacological Laboratory, Johns Hopkins University, Baltimore, Md.*]

A phyto-pharmacological study was carried out much in the same way as the effects of cocaine were studied by Macht and Livingston. The influence of a number of isomeric drugs was studied on the roots of *lupinus albus*. The following structural chemical isomers were examined: normal propyl alcohol and isopropyl alcohol, primary butyl alcohol and secondary butyl alcohol; primary amyl alcohol and secondary amyl alcohol. It was found in every case that the normal or primary alcohols were more toxic to the growth of the lupine than the corresponding secondary alcohols.

The following stereo-isomers were examined: laevogyrous camphor, dextrogyrous camphor and optically inactive camphor; quinin and quinidin; cinchonin and cinchonidin. It was found that laevogyrous camphor was more toxic than the dextro variety, while the inactive camphor produced an effect equivalent to the mean of the other two. Quinin (sulphate) which is laevogyrous was found to be more toxic than quinidin (sulphate) which is its dextrogyrous isomer. In the same way cinchonidin (sulphate) which is laevogyrous was more toxic than cinchonin (sulphate) which is its dextrogyrous isomer.

The difference in toxicity between the various forms of camphor is also illustrated by the effect of solutions of these drugs on the growth of moulds and bacteria. Aqueous solutions of the three varieties of camphor were made and exposed to the air. It was found that the dextro-camphor solution very soon became cloudy. The solution of inactive camphor became contaminated with bacteria and moulds some time later, while the solution of laevogyrous camphor remained clear for a very long time. The relative toxicity of both the primary and secondary alcohols and the various stereo-isomers which were studied corresponds to the relative toxicity of the same drugs for animals tissues as studied by the author.

12 (1972)

The supposed relation of the adrenals to reflex volume changes in the denervated limb.

By G. N. STEWART and J. M. ROGOFF

[*From the H. K. Cushing Laboratory of Experimental Medicine, Western Reserve University, Cleveland, Ohio*]

Bayliss[1] showed that when the central end of a peripheral nerve (sciatic) is stimulated the volume of a hind limb (denervated by section of the anterior crural and sciatic nerves) increases coincidently with the rise of blood pressure and then diminishes to less than the initial volume. He explained the dilatation of the limb as a passive dilatation due to the increase of blood pressure and the subsequent constriction as a local reaction, "the muscular coat of the arteries reacting, like smooth muscle in other situations, to a stretching force by contraction."

Von Anrep[2] stated that constriction of the limb never occurred in the absence of the adrenals. He drew the conclusion that it is due to a reflex increase in the epinephrin output, and that the explanation of Bayliss was not satisfactory.

We have reinvestigated the question in a series of about 50 animals, chiefly dogs.

We find that a typical reaction can be elicited in acute experiments after the adrenals have been clipped or tied or the glands excised, in the great majority of dogs in which before interference with the adrenals the reaction was present. In a certain number of animals, the reaction has not been obtained by us either before or after interference with the adrenals. Bayliss[3] seems also to have encountered animals in which similar reactions were not obtainable.

A considerable number of experiments were made on dogs which had recovered from adrenal operations entailing marked interference with, or suppression of the epinephrin output (removal of one adrenal and the greater part of the other, with curetting away of the remaining medulla and denervation of the

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[1] Bayliss, *Journ. of Physiol.*, 1902, xxviii, 220.

[2] Anrep, *Journ. of Physiol.*, 1912-1913, xlv, 307, 318.

[3] Bayliss, *Journ. of Physiol.*, 1902, xxviii, 288.

fragment). Even after removal of the adrenal remnant typical reactions were obtained.

We must accordingly conclude that Anrep's experiments do not constitute a proof that the epinephrin output is reflexly increased by stimulation of the central end of the sciatic.

Stimulation of the splanchnic, with either the corresponding or both adrenals eliminated, frequently gave good reactions. We do not doubt, however, that since splanchnic stimulation is known to increase the epinephrin output, such increase with direct stimulation of the peripheral secretory nerves, when the adrenals are intact, can be a factor in the reaction as obtained in this way.

13 (1973)

Lasting individual differences in the resistance of normal bloods to shaking.

By OSWALD H. ROBERTSON and PEYTON ROUS

[*From the Rockefeller Institute, New York City*]

In previous papers observations have been recorded which indicate that the normal destruction of red cells is accomplished, in part at least, by a fragmentation of the elements while circulating[1]. It has seemed possible that the behavior of cells shaken in *vitro* may yield some indication of their resistance to the fragmenting process.

Marked differences in the red cells from different species have already been disclosed by the shaking method[2]. Further observations have now been made. Shaking which suffices to liberate 10-25 per cent. of the hemoglobin contained in a suspension of washed cells of the rat brings out only 4 per cent. of the pigment from an average specimen of rabbit cells, 1 per cent. from monkey blood, and a mere trace from human blood. Dog corpuscles are among the most labile, as many investigators can attest who have striven to obtain plasma untinted by hemolysis.

The variation in the resistance of individual bloods of a single species, the rabbit for example, are by no means inconsiderable.

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[1] Rous, Peyton, and Robertson, O. H., *Jour. Exper. Med.*, 1917, xxv, 651.

[2] Rous, Peyton, and Turner, J. R., *Jour. Exper. Med.*, 1916, xxiii, 219 and 239.

The method of washing and shaking the cells has already been described. Healthy adult animals with bloods showing the same general ratio of hemoglobin to corpuscle bulk were employed. The amount of hemoglobin set free was read off as acid hematin by comparison with a standard series of tubes containing graded solutions of the substance. In one experiment washed specimens from 20 rabbits, shaken at the same time, yielded from 1.2 per cent. to 5.4 per cent. of their hemoglobin; in another set of 27 individuals, 0.8 per cent. to 5.7 per cent. was set free. The range of resistance here exhibited was in striking contrast to the uniformity of the response to a specific hemolysin in graded dilution. The resistance to hypotonic salt solution, while much less uniform than this latter, was still not so frequently variable as that to shaking, though occasional instances of marked individual susceptibility were encountered. Generally speaking, resistance to the one means of cell injury yielded no indication of that to the other.

The individual blood differences disclosed by the shaking method persisted throughout the period of our observations (43 days). They were independent of sex, weight, normal variations in bone-marrow activity as indicated by the percentage of circulating reticulocytes, and of moderate intercurrent changes in the hemoglobin percentage and in the number of red cells per cu. mm. of blood. They did not tally with the individual variations in the rate of breaking down of artificial subcutaneous extravasates. The spleens of the individuals with fragile cells proved to be no larger, and erythro-phagocytosis was no more marked than in the animals with resistant cells. The organs of all were normal.

It would be difficult to employ the resistance of the blood to shaking as a clinical test. Pathological variations in the pigment-stroma ratio, and in corpuscle size are among the more serious complicating obstacles.

14 (1974)

The penetration of arsenic into living cells[1].

By MATILDA MOLDENHAUER BROOKS (by invitation)

[*From the Division of Pharmacology, Hygienic Laboratory, Washington, D. C.*]

The purpose of these experiments was to study directly the penetration of arsenic into living cells. The organism used was the fresh water alga *Nitella,* which furnishes single cells several inches in length, whose contents may be easily expressed and analysed. Freshly collected cells only were used.

To determine whether there is any relation between H ion concentration and arsenic penetration ten .033 M phosphate buffer solutions were made up covering the range P_H 5.6 to 7.5, and at least twenty-five cells of *Nitella* placed in each of these for each experiment. After 24 hours counts were made of the number of cells still in good condition, *i.e.,* turgid and with cell sap clear and free from chloroplasts.

A series of similar experiments was performed in which arsenic in the form of atoxyl was added to the buffer solutions in sufficient quantity to make the concentration of atoxyl .05 M; and still another series in which a set of 0.05 M arsenate buffers was used (arsenic acid plus NaOH) having the same P_H range as the phosphate buffers. In each case the proportion of cells of *Nitella* still in good condition after 24 hours in the solutions was determined.

In both experiments with arsenic the sap of all the cells in good condition was expressed (precautions for preventing contamination being taken), a constant quantity (0.036 c.c.) incinerated, and the arsenic determined by the Gutzeit method, which is sensitive to about one micromilligram.

All experiments were conducted at laboratory temperature, which was usually not far from 20° C.

1. The influence of both hydrogen ions and buffer salt ions upon the condition of *Nitella* is shown by the facts that:

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[1] Approved for publication by the Surgeon General, U. S. Public Health Service.

a. In phosphate buffers of .033 M the proportion of cells surviving in good condition was greatest, (92 per cent.) at P_H 6.2, and decreased when the medium was more acid or alkaline.

b. When atoxyl (in .05 M concentration) was present the number of cells in good condition after 24 hours was greatest, (94 per cent.) at P_H 5.6, decreased gradually to 86 per cent. at P_H 6.4 and then suddenly to 6 per cent. at P_H 7.5.

c. Arsenate buffers (of .05 M) acted much like phosphate buffers, but showed a greater general toxicity: thus under the most favorable conditions (P_H 6.4) 80 per cent. of the cells survived in good condition as compared with 92 per cent. in the case of the phosphate buffers.

d. The optimum P_H for survival of *Nitella* when placed in either phosphate buffers or arsenic buffers was 6.2-6.4, and was not the same as the normal P_H of the cell sap of *Nitella,* which was found to be 5.7.

2. The conditions affecting arsenic penetration are illustrated by the experiments with solutions containing atoxyl, in which no arsenic penetrations was observed between P_H 5.6 and 6.4, while positive tests were obtained at all higher values of the P_H with a maximum of 22 mg. in 0.036 c.c. of cell sap at P_H 7.5, which was the most alkaline solution used.

3. The amount of arsenic in living (even though perhaps injured) cells was always considerably less than the arsenic content of the solution in which the cells were placed.

4. Dead cells, *i.e.,* those which were limp and with shrunken cell contents had an arsenic content approximately equal to that of the solution in which they were placed.

5. The cell walls were found to contain considerably more arsenic than the cell sap.

From these experiments it may be concluded that the penetration of the arsenic of atoxyl into the cell sap of *Nitella* is strikingly correlated with the H ion concentration of the suspension fluid. This apparent relationship between the H ion concentration of the suspension fluid and the penetration of arsenic may possibly be due to (1) an influence of the H ion concentration upon the dissociation of atoxyl, or (2) changes in the permeability of the cell for arsenic due to the action of either buffer ions or H ions.

15 (1975)

Observations on the relation of the adrenal glands to the blood-pressure response during cerebral anæmia in cats and rabbits.

By HELEN C. COOMBS and J. M. ROGOFF

[*From the Department of Experimental Medicine, Western Reserve University, Cleveland, Ohio*]

The blood-pressure response during cerebral anæmia was analyzed by Pike, Guthrie and Stewart a number of years ago. Recently Winkin in studying some of the nervous factors involved in the cardio-vascular changes which take place during cerebral anæmia observed that after repeated occlusions of the head arteries for short periods, the curve of the anæmic rise may become dissociated into two distinct parts.

The question was raised by Winkin whether the second part of this anæmic rise may not be due to increased availability of some product of adrenal activity which the "cardio-vascular relations found in the mammalian organism under extreme conditions of stress" would call forth. With this in mind, we have carried out a series of experiments on cats and rabbits when:

1.   The adrenal glands are tied off, or excised during an acute experiment.

2.   The adrenal veins are clipped.

3.   The remaining adrenal is excised (one having been previously excised and the animal allowed to recover).

4.   One adrenal is excised and the other denervated (and the medulla of it curetted out or a large part of the remaining gland excised in addition to denervation) and the animal allowed to recover.

5.:   Both adrenals are excised (rabbit) and the animal allowed to recover.

In cases 3, 4, and 5 the animals were operated upon from two to four weeks before the acute experiments were performed. The technique of the acute experiment was that devised by Stewart, Guthrie, and Pike in which the arteries are secured as they emerge from the thorax. It is well known that in the cat and rabbit, when the carotid and subclavian arteries (proximal to the origin of the vertebrals) are occluded, circulation to the head

is completely interfered with and cerebral anæmia with the attendant anæmic rise of blood-pressure ensues rapidly. The high level of blood-pressure is maintained until the fall to the spinal level of pressure indicates the failure of bulbar function. If the occlusion has not been carried on for too long a time, release of the head arteries with the maintenance of artificial respiration results, eventually, in restoration of the bulbar and cerebral function, the degree of restoration of function depending on the amount of injury that has been inflicted by the occlusion.

In our experiments, the beginning of the fall in blood-pressure, after the anæmic rise induced by occluding the head arteries, was the signal for the restoration of cerebral circulation, and the return of the blood-pressure to the previous level (or nearly so), together with the return of an active corneal reflex were usually the criteria for the beginning of the next occlusion of the head arteries. Artificial respiration was constantly maintained after the beginning of the first occlusion.

By employing this technique a number of occlusions (up to about thirty) can be made before there is a lack of response from the medulla to cerebral anæmia. In a large number of cases, after the first few occlusions, the curve of the anæmic rise has been observed to dissociate into two parts, each occupying about half the time which an undissociated curve would occupy, as reported by Mrs. Winkin. This type of dissociation curve we have obtained, however, not only in normal animals, but with equal success in the animals in which epinephrin secretion had been suppressed or abolished.

Moreover, animals in which adrenal function had been greatly interfered with or abolished by the operations above mentioned are able to respond in the usual manner to as many occlusions of the head arteries as normal animals. We have found that the number of definite responses to cerebral anæmia is largely dependent upon the general blood-pressure level existing just before the occlusion and that when this has fallen to spinal level so that no rise in blood-pressure can be obtained on occlusion of the head arteries, it is possible to obtain good responses by raising the basal level through injection of Ringer's solution, so long as the Ringer's solution is capable of sustaining a higher level of blood-pressure. But when a condition is reached when the blood-pressure improvement is transient, the usual responses are

no longer obtainable on occlusion of the arteries. Resuscitation can frequently be repeated with Ringer's solution a number of times before a condition is reached when it is no longer effective.

16 (1976)

Barium-epinephrin antagonism on the excised surviving intestine.

By GEORGE B. ROTH

[*From the Pharmacological Laboratory of the Medical School, Western Reserve University, Cleveland Ohio*]

Experiments conducted recently on the excised surviving intestine of the frog, showed that the barium contraction could be wholly or partially removed by epinephrin (Arch. Inter. de Pharmacodyn. et de Therap. Paper in press).

Inasmuch as the frog's intestine in Tyrode's solution, reacted to pilocarpine in an unexpected, heretofore undescribed manner, namely, to produce relaxation, it was thought that the barium-epinephrin antagonism was peculiar to the frog.

Further experiments, in which excised surviving intestinal segments, from the turtle and rabbit were used, showed that the barium-epinephrin antagonism could be demonstrated in these animals. For example, in the turtle, the contraction caused by 10 mg. of barium chloride was completely antagonized by 0.4 mg. of epinephrin. This is, of course, contrary to the current conception that barium act, directly on the contractile substance and epinephrin on the receptive mechanism.

This antagonism does not seem to have been previously described in the literature; however, Professor A. N. Richards informs me that he also had observed it in the excised rabbit's intestine.

17 (1977)

The effect of change in type of intestinal bacteria on urinary indican and phenols.

By ARTHUR H. SMITH and W. L. KULP

[*From the Sheffield Laboratory of Physiological Chemistry and the Bacteriological Department, Yale University, New Haven*]

Experiments were carried out on seven individuals to determine the effect on urinary indican and phenols brought about by change from essentially putrefactive to aciduric type of intestinal flora. In the experimental period one quart of a skim milk culture of *B. acidophilus* containing 100 grams lactose was added to the ordinary diet. Total nitrogen, indican, free and combined phenols were determined in the urine and the bacterial count was made on the feces.

Two of the subjects failed to react to change of flora under the treatment. Three others showed a tendency toward decrease in urinary indican and combined phenols when *B. acidophilus* attained a concentration of 90-100 per cent. in the feces. The remaining two showed increased indican and phenol excretion when the milk culture was added to the diet although the concentration of *B. acidophilus* in the feces was at least 90 per cent.

These data suggest that, when *B. acidophilus* is given in milk culture, in some subjects the increased amount of available tryptophane more than balances the decrease in numbers of indol-producing organisms with the result, that increased ethereal sulfate output may accompany a preponderatingly aciduric intestinal flora. It is likewise indicated that the favorable clinical results obtained with milk cultures of *B. acidophilus* in gastro-intestinal cases do not depend primarily on decreased production of indican and ethereal sulfates.[1]

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[1] Part of the bacteriological examinations was made by Dr. H. A. Cheplin.

18 (1978)

The influence of diet and of B. acidophilus ingestion on intestinal putrefaction.

By LUDWIG KAST, JAMES J. SHORT and HILDA M. CROLL

[*From the Departments of Medicine and Biochemistry, New York Post-Graduate Medical School and Hospital, New York City*]

A series of about two hundred and fifty cases, including normal individuals, a large number of patients representing a variety of pathological conditions, and a group of patients suffering from symptoms supposedly due to intestinal disorders such as constipation, autointoxication, colitis, arthritis, eczema and urticaria, have been treated by dietary restrictions affecting the protein foods. About six hundred and fifty quantitative determinations of the indican, phenols, total nitrogen and creatinine excreted in the twenty-four hour urines have been made. It has been found that a decrease in the total nitrogen intake (meat and eggs) results in a corresponding decrease in the putrefactive products excreted by the same patients when on a higher protein diet. The phenols, as determined by the method of Folin and Denis[1], however, showed much less tendency toward significant changes due to diet than did indican. In as much as Tisdall[2] has suggested the non-specificity of this method for determining phenols, our results would lend support to the conclusion that this method for phenol estimation is of little value.

The effect of feeding one liter of *B. acidophilus* milk, prepared according to Rettger and Cheplin[3] from Rettger's cultures, and 100 grams of milk sugar daily, in addition to ordinary diets, was studied in eight patients suffering from constipation, eczema and colitis. No milk was allowed in the diet on the control days previous to the feeding of the acidophilus milk. The absolute amount of protein in the daily diet was kept as nearly constant as possible throughout the periods of observation, although there was of necessity a decrease in certain protein foods to allow for

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[1] Folin, O., and Denis, W., *J. Biol. Chem.*, 1915, xxii, 305.

[2] Tisdall, F. F., *J. Biol. Chem.*, 1920, xliv, 409.

[3] Rettger, L. F., and Cheplin, H. A., *The Intestinal Flora*, Yale University Press, 1921.

that ingested in the milk.   The time required to bring about practically complete transformation of the intestinal flora to the acidophilus type varied with the individuals studied, from several days to several weeks.   This observation is based on frequent cultural examinations of the stools according to Rettger's technique.   In general, the excretion of indican (and of phenols to a less extent), increased early in the treatment with acidophilus, followed by a gradual lowering as the treatment continued; the amounts of these products did not usually fall below those excreted before the acidophilus feeding.   If indican excretion may be taken as an index of intestinal putrefaction, it appears that implantation of *B. acidophilus* in the intestine does not necessarily lower putrefactive processes in the intestine.

19 (1979)

Is cystin synthesized in the animal body?

By J. A. MULDOON, G. J. SHIPLE and C. P. SHERWIN

[*From the Chemical Research Laboratory, Fordham University, New York City*]

We undertook a series of experiments in order to determine which of the amino acids occurring in proteins were possible of synthesis in the animal organism.   According to Abderhalden, any of the aliphatic amino acids should be synthesized, but probably none of the hetero-cyclic or aromatic acids.   It has already been shown that glycocoll can be built in the animal organism[1] [2] and we have previously shown[3] that glycocoll and also glutamine can be synthesized in the human body at the expense of nitrogen which would otherwise be found in the urea portion of the urine, and peculiarly that both acids are prepared simultaneously as well as singly.

Recent feeding experiments have shown that cystin is a necessary amino acid in protein if growth or maintenance of body weight is desired, therefore indicating that neither cystin nor cystein is synthesized in the organism.

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[1] McCollum, E. V., and Hoogland, D., *Jour. Biol. Chem.*, 1913, xvi, 311.

[2] Lewis, H. B., *Jour. Biol. Chem.*, 1914, xviii, 225.

[3] Shiple, G., and Sherwin, C. P., *Jour. Amer. Chem. Soc.*, 1922, xliv, 618.

In 1879 Baumann[4] and Jaffe[5] independently discovered that brom-benzene fed to dogs is detoxicated by joining the cystein molecule followed by an acetylation of the amino group of the latter.

We thought that perhaps under these conditions of brom-benzene intoxication it might be possible for the organism to synthesize cystein, although it was unable to do so for dietary purposes.

Kopfhammer[6] at this time published a paper showing that on a non-protein diet the dog is unable to synthesize cystein, although at the same time fed brom-benzene. We then undertook three series of experiments. In the first we placed the dog on a carbohydrate diet and fed each day brom-benzene. Besides this on the first two days we fed sodium sulphate, the second two days we fed taurine, the next two days calcium sulfate, the following two days sodium sulphocyanate, then following this ethylamino mercaptan, and finally cystin. Thus the dog was furnished more than sufficient sulphur in many different forms but was unable to use it for the elaboration of cystein. Only when cystin was fed was there a detoxication of brom-benzene in the manner described above. The second experiment was a repetition of the first but added to this was sufficient ammonium acetate to furnish inorganic nitrogen for the amino group of cystein, but still no synthesis of this amino acid. The same results were obtained in a third experiment which was a repetition of the second, only gelatine was added to furnish the organic nitrogen instead of the inorganic nitrogen.

It is safe to say therefore that cystein and consequently cystin can not be synthesized in the animal body even under the strain of brom-benzene poisoning when inorganic or organic nitrogen is furnished for the amino nitrogen and different forms of sulphur for the sulphydril group.

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[4] Baumann, E. and Preusse, C., *Ber. chem. Gesellsch.*, 1879, xii, 806.

[5] Jaffé, M., *Ber. chem. Gesellsch*, 1879, xii, 1092.

[6] Kopfhammer, J., *Zeit. Physiol. Chem.*, 1921, cxvi, 302.

20 (1980)

On the nature of the anti-tryptic action of serum and its biologic significance.

By ALBERT A. EPSTEIN

[*From the Department of Physiological Chemistry, Mt. Sinai Hospital, New York City*]

The power which serum possesses to inhibit tryptic digestion is a well known phenomenon, but the nature of the process is not well understood. This is evidenced by the fact that the anti-tryptic power of serum has been variously ascribed to the proteins, the lipoids, and the nitrogenous crystalloids of the serum, and more recently to the saponified unsaturated fatty acids present in it.

The observations to be reported were made in the course of a study begun two years ago on the isolation of the different pancreatic ferments. For reasons which will be discussed at another time, the anti-tryptic action of serum was investigated. At first an inquiry was made into the particular substance in the serum which was endowed with the power to inhibit tryptic digestion. Our results were in conformity with those who found that the albumin fraction of the serum proteins was chiefly responsible for the phenomenon. It may be added that other substances contribute to the total inhibitory action of serum. The globulins, for example, possess the power to a limited degree, and only when present in concentration. While studying this phenomenon with whole serum, in great dilution, it was found that the degree of inhibitory action was proportionate to the quantity of albumin present. Under such conditions other substances apparently played no part in the reaction. The inhibitory action obtained from the fatty substances present in the serum is insignificant, when contrasted with the remarkable power which whole serum or its albumin fraction possesses.

The next question investigated was the nature of the inhibitory action. You will recall that when the anti-tryptic action of serum was first observed it was thought to resemble the action of the specific anti-body of the immune system. This view was soon abandoned and the multiplicity of opinions concerning the

particular substance which causes the inhibition, existing at present, left this question entirely unsettled.

The first point considered was the fate of trypsin in mixtures with serum. Several effects seemed possible; (1) that trypsin was destroyed by the serum and that the action was purely toxic; (2) that the reaction between trypsin and serum was one of physical adsorption; and (3) that the reaction might be of a chemical nature.

In some previous work reported before this society[1], it was found that trypsin could be easily isolated from fresh and commercial extracts of the pancreas in a state of great purity by means of colloidal iron. It seemed possible, therefore, that this method of isolating the ferment might prove of service in studying the problem in hand; and indeed such was the case.

By means of adequately chosen experiments it was found that trypsin could be recovered quantitatively from mixtures with serum even after prolonged periods of incubation. Other means of isolating trypsin were also found, for example, by treating mixtures of trypsin and serum with adequate amounts of ethyl alcohol, in which case the proteins of the serum are precipitated and the trypsin remains in solution. It might be added that the latter method is not quantitative.

These experiments indicate, I believe, the following: that the anti-tryptic action of serum is not due to a destruction of the ferment by the serum, but that the inhibitory action of serum is either due to an adsorption of the trypsin by the serum, or that the reaction is a chemical one, of a reversible nature.

When mixtures of trypsin and a digestible substrate such as casein are treated with alcohol, after a brief incubation, some of the trypsin can be recovered. After digestion of the casein is completed none of the trypsin can be recovered. When such mixtures are treated with colloidal iron the trypsin can not be isolated by reason of the fact that the reaction takes place between the casein and the iron, forming casein-ferrate. When, however, serum is first added to a mixture of trypsin and casein, in sufficient amount to cause inhibition of digestion, and this is then treated with colloidal iron, both the serum proteins and the casein are precipitated and the trypsin remains in solution. The

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[1] Epstein, Albert A., Proc. Soc. Exper. Biol. and Med., 1921, xix, 3.

same result is obtained when such mixtures are treated with ethyl alcohol. These experiments justify the conclusion that the inhibitory action of serum on trypsin is in the nature of an interference phenomenon. That is to say, that the serum protein, either by virtue of a chemical reaction with the casein or a simple mechanical process acts as a protective colloid. This conclusion, however, does not entirely rule out the possibility that a chemical reaction takes place between trypsin and serum which is readily dissociable.

In the communication on trypsin alluded to above[1], I stated that the ferment appears to be of a strongly acid character. As is known it has a positive electric charge, whereas serum albumin which is chiefly concerned in the anti-tryptic phenomenon, is said to have a negative charge, so that a reaction between the two is conceivable.

As to the biologic significance of the anti-tryptic action of serum, I will say only a few words at the present time. Serum possesses diastatic and lipolytic ferments; tryptic ferments on the other hand are not ordinarily demonstrable. Because of the anti-tryptic action, failure to demonstrate trypsin in serum does not necessarily imply that no trypsin is present. However, by the methods suggested trypsin can be recovered from serum. Therefore the question arises as to the source of all these ferments and the relation which the pancreas bears to them. There are two possibilities, namely: (1) that the ferments arise from the pancreas, and (2) that they arise from the tissues and that the pancreas serves as an organ of excretion. This phase of the subject will be taken up at another time.

ABSTRACTS OF COMMUNICATIONS, PACIFIC COAST BRANCH

Thirty-fourth meeting.

Berkeley, California, October 15, 1922

21 (1981)

Retardation of metamorphosis in the Colorado axolotl by the intraperitoneal injection of fresh bovine hypophyseal anterior lobe substance[1].

By PHILIP E. SMITH and IRENE B. SMITH

[*From the Anatomical Laboratory, University of California, Berkeley, California*]

As is well known, the axolotl in its native habitat may retain its larval condition for long periods, even becoming sexually mature (neotonous). When treated with thyroid, when placed in unfavorable conditions or when transported to a lower and warmer region it rather promptly metamorphoses. This delicate balance obtaining in the internal secretory glands (the thyroid and hypophysis here being of especial interest) of this form would appear to make it especially useful in the experimental modification of the activities of these glands. Since the experimental transplantation of the anuran anterior hypophysis into the hypophysectomized tadpole (Allen) and into the normal tadpole (Swingle) and the intraperitoneal injection of bovine anterior hypophyseal substance into the pituitaryless tadpole (Smith) appear to stimulate the thyroid, thus hastening or inducing metamorphosis, it would seem that the injection of this substance might be expected to accelerate metamorphosis in the axolotl. *The opposite reaction, however, is evoked, the larval condition being decisively prolonged.* The experimental injection of fresh bovine anterior lobe hypophyseal substance, intraperitoneally, into the Colorado axolotl during the months of May to September of the present year has resulted in a definite retardation in the metamorphosis of this form. Anterior lobe substance appears here to have given a "paradoxical" reaction and the usual effect of thyroid activity (which most investigators

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hold as immediately responsible for metamorphosis) has been greatly postponed. A pronounced darkening of the axolotl resulted from these injections of anterior lobe substance, the specimen becoming after repeated injections a jet black

22 (1982)

Reactions of the capillary endothelium in peptone shock.

By W. H. MANWARING, WALTER H. BOYD and WILLIAM O. FRENCH

[From the Laboratory of Experimental Pathology, Stanford University, San Francisco, California]

The fundamental reacting tissues in peptone shock are not the same as the fundamental reacting tissues in the acute anaphylactic shock of dogs. Canine anaphylactic shock is dependent upon liver function[1]. Canine peptone shock is not dependent upon hepatic function, since reactions apparently identical with those of the intact animal are produced by intravenous injections of peptone into dehepatized (Dale and Laidlaw's Eck-fistula[2]) dogs and into eviscerated dogs.

Marked peptone reactions are demonstrable in isolated canine tissues. These reactions are produced by perfusing the tissues with Ringer's solution containing 1 per cent. Witte's peptone. More marked reactions are obtained by perfusing with defibrinated-blood-peptone mixtures, or with uncoagulated-blood-peptone mixtures. The principal reactions of the isolated canine tissues thus far studied are:

(a) *Liver.* Marked increase in perfusion resistance, reaching a maximum by the end of ninety seconds. The resistance then gradually decreases, and is almost completely restored to normal by the end of eight minutes.

(b) *Lungs.* Reactions similar to those of the liver, but more pronounced, with little or no tendency to recovery by the end of eight minutes.

(c) *Intestines.* Distinct decrease in perfusion resistance, reaching a maximum by the end of ninety seconds. Slight tendency to recovery by the end of eight minutes.

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[1] W. H. Manwaring, *Zeitschr. f. Immunitätsf.*, 1911, viii, 1.

[2] H. H. Dale and P. P. Laidlaw, *Jour. Physiol.*, 1918-19, lii, 351.

(d) *Hind Quarters.* Reactions similar to those of the intestines, but with less tendency to recovery by the end of eight minutes.

We have endeavored to determine the mechanism of the changed perfusion resistance by histological methods. The tissues have been fixed by perfusion methods at various stages of the peptone reaction. The following appear to be the dominant physiological factors thus far studied:

(a) *Liver.* Marked capillary vaso-constriction with stasis and diapedesis in certain areas. Partial capillary occlusion by leucocytic deposits in later stages of the shock. Marked increase in tissue lymph, with dilation and even rupture of the lymphatics, edema of the connective tissue structures, and mechanical separation of the capillary endothelium from the parenchymatous cells. Swelling and vacuolization of the parenchymatous cells.

(b) *Lungs.* Marked capillary vaso-constriction.

From these observations we believe that the dominant circulatory factors in canine peptone shock are reactions of the capillary endothelium. According to this conception the fundamental physiological reactions in peptone shock are:

(a) Pulmonary and hepatic capillary vaso-constriction.

(b) Capillary vaso-dilation in other parts of the body.

(c) Increased capillary permeability most marked in the liver.

The reactions in the extra-hepatic and extra-pulmonary capillaries are similar to the endothelial reactions recently described by Dale and Laidlaw[3], Rich[4], and others in histamine shock.

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[3] H. H. Dale and P. P. Laidlaw, *Jour. Physiol.*, 1918-19, lii, 355.

[4] A. R. Rich, *Jour. Exper. Med.*, 1921, xxxiii, 287.

23 (1983)

Study of bacterial toxins by means of the isolated mammalian heart.

By W. H. MANWARING and WALTER H. BOYD (by invitation)

[*From the Laboratory of Experimental Pathology, Stanford University, San Francisco, California*]

The excised rabbit heart is a delicate reacting index of toxic action. It is well suited to the study of bacterial products giving little or no recognizable reaction with intact animals. The technique we have used is the simplified technique proposed by Gunn[1]. The preferable perfusion fluid is Locke's solution containing 1 per cent. carefully filtered defibrinated rabbit blood. Control hearts perfused with this mixture beat regularly and strongly for over three hours. With hemolytic or hemagglutinating bacterial products this blood mixture, of course, can not be used. With such products, Locke's solution alone may be used, or Locke's solution containing 0.1 per cent. carefully filtered laked blood. Perfused with these solutions, the isolated rabbit heart beats regularly and strongly for over an hour. Illustrative cardiac reactions are given below:

1. *Endotheliotoxin of S. cholerae.* Filtrates from broth cultures of *S. choleræ* are almost non-toxic for the contractile and conducting tissues of the isolated rabbit heart. Minor changes in tone, and in rate and strength of contraction are produced, but the hearts beat regularly and strongly for over ninety minutes.

Very marked reactions on the capillary endothelium, however, are produced. Within fifteen minutes, the myocardium becomes markedly edematous and markedly hemorrhagic. Histological sections show the tissue spaces widely dilated, and containing numerous extravasated red blood corpuscles. It is believed that this reaction furnishes a valuable method for the study of the immunological adaptations of the capillary endothelium.

2. *Hemagglutinin of Streptococcus hemolyticus*: The streptococcus was grown in a 10 per cent. dilution of defibrinated rabbit blood in Locke's solution[2]. On testing these filtrates with

1 J. A. Gunn, *Jour. Physiol.*, 1913, xlvi, 506.

2 A. H. Clark and L. D. Felton, *J. A. M. A.*, 1918, lxxi, 1048.

1 per cent. blood-Locke-mixtures, a very rapid decrease in the
rate of perfusion flow was noted. The coronary perfusion al-
most ceased by the end of five minutes.

Histological sections of hearts thus perfused show an almost
total occlusion of the cardiac arterioles with agglutinated red
blood corpuscles. In most of our parallel test tube experiments,
recognizable hemagglutination did not take place. It is believed
that cardiac perfusion furnishes a more delicate index of hemag-
glutination than the ordinary test tube reaction[3], and throws im-
portant light on the mechanism of streptococcus pathogenicity.

3 *Cardiotoxin of Streptococcus hemolyticus*: In corpuscle-
free perfusions, streptococcus filtrates are markedly toxic for
the contractile and conducting tissues of the isolated rabbit
heart. The filtrates uniformly produce: (i) a marked loss of
myocardial tone, usually reaching a maximum within three
minutes, (ii) a temporary complete auricular-ventricular dis-
sociation (heart-block) usually lasting about five minutes, and
(iii) a progressively decreasing strength of the myocardial con-
tractions, usually leading to complete cessation of recordable
movements in from twelve to fifteen minutes.

It is believed that these reactions throw light on the mechanism
of streptococcus toxicity, and give a valuable method for the
study of the immunological adaptations of cardian tissues.

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3 K. M. Howell, *Jour. Infect. Dis.*, 1920, xxvii, 565.

24 (1984)

The relative therapeutic efficiency of arsphenamine and gelatin-arsphenamine[1].

By JEAN OLIVER

[*From the Department of Pathology of the Medical School, Stanford University, San Francisco, California*]

Under certain conditions of H ion concentration compounds are formed between di-sodium arsphenamine and hydrophile colloids. The amount of arsphenamine bound varies with the nature of the colloid and the P_H of the medium in which the reaction occurs. The relative affinity of arsphenamine for certain of these colloids, stated in a descending series, is: gelatin, globulins, gum arabic and egg albumin. A similar union between arsphenamine and the plasma proteins, especially the globulins, may be demonstrated *in vivo* following the intravenous administration of arsphenamine, and it may be further shown that this union is the mechanism by which the animal is protected from the agglutinating action which arsphenamine shows towards the red cells of the blood. If a large amount of arsphenamine is administered, the plasma proteins are "saturated," free arsphenamine is bound by the red cells and agglutination of them results. Under such conditions the compound of plasma globulins, including fibrinogen, with arsphenamine, is found to be no longer coagulable by either heat or thrombin. The shed whole blood from such an animal does not coagulate on standing[2].

From these facts it would seem that the administration of arsphenamine previously bound to such a colloid as gelatin would augment the protecting factors and result in a lower toxicity of the drug. As we have reported[3] such is found to be the case. The immediate or physical toxicity, due to embolism from agglutinated red cells, is lowered to such a degree that relatively enormous doses are well borne. The late, or chemical, toxicity

[3] Proc. Soc. Exp. Biol. and Med., 1922, xix, 304.

is also lowered, the ratio of the minimum lethal dose of arsphenamine to that of gelatin-arsphenamine being as 10 is to 14.

The relative therapeutic action of arsphenamine and gelatin-arsphenamine was next examined. A priori it may be argued that the gelatin will "protect" the parasite in the same manner as it does the blood cells and tissues of the host. On the other hand, our experiments have shown that the gelatin-arsphenamine is more slowly excreted, and that therefore a higher concentration of it persists in the blood stream and tissues than after the administration of an equal amount of arsphenamine. The therapeutic efficiency of gelatin-arsphenamine would be the resultant of these two opposing factors.

In testing the therapeutic efficiency we have followed the method described by Voegtlin[4]. *Trypanosoma Brucei* were used. Rats showing from 150,000 to 300,000 trypanosomes per c.mm. of blood were given increasing doses of the two substances intravenously. The blood was examined after 24 hours for the presence of parasites and every day for 3 weeks.

The result of 50 experiments may be summarized as follows: Arsphenamine and gelatin-arsphenamine have the same minimum effective dose of 10 mg. per kilo. Such a dose frees the blood stream of parasites in 24 hours. The minimum sterilizing[4] dose[5] which prevents relapses for 21 days is also the same with the two drugs, 20 mg. per kilo.

The chart compares these findings to the results of similar experiments of Voegtlin and of Schamberg, Kolmer and Raiziss with arsphenamine and neo-arsphenamine. It will be noted in comparing the minimum effective doses that the ratio of the indices of therapeutic efficiency, $\dfrac{\text{minimum lethal dose}}{\text{minimum effective dose}}$, of gelatin-arsphenamine is 1.4 times greater than that of arsphenamine. As will be seen under Voegtlin's findings, this figure is slightly larger than the corresponding ratio of neo-arsphenamine to arsphenamine. Gram for gram, however, gelatin-arsphenamine is twice as effective as neo-arsphenamine and equally effective as arsphenamine.

[4] *Public Health Reports*, 1922, xxxvii, 1627.

[5] Schamberg, Kolmer and Raiziss, *Amer. Journ. Med. Sciences*, 1920, clx, 25.

The minimum sterilizing dose gives similar results. The therapeutic index $\dfrac{\text{maximum tolerated dose}}{\text{minimum sterilizing dose}}$, of gelatin-arsphenamine is 1.4 times that of arsphenamine and corresponds in this regard to Kolmer's findings with neo-arsphenamine. Gram for gram, gelatin-arsphenamine is almost twice as effective as neo-arsphenamine.

These relations may be summarized as follows: Gelatin-arsphenamine is as effective as arsphenamine and 5/7 as toxic. It is 1.9 times as toxic as neo-arsphenamine, arsphenamine being 2.4 times, and twice as effective.

As used above "toxicity" refers to the late chemical toxicity of the drug. An even more striking contrast is shown if the immediate physical toxicity of arsphenamine and gelatin-arsphenamine is considered. Considerable evidence has been advanced indicating that the "reactions" occurring during or soon after the injection of arsphenamine are due to this physical toxicity[6][7]. Practically speaking, gelatin-arsphenamine has no immediate toxicity. The immediate toxicity of arsphenamine varies with the condition of the individual. For these reasons no exact ratios can be established, but it can be stated that whereas on account of its physical toxicity, a resulting "acute reaction" is always a possibility during the injection of arsphenamine, it seems unlikely from the experimental evidence on hand that such acute mishaps could occur with gelatin-arsphenamine under any conditions or with any dosage.

MINIMUM EFFECTIVE DOSE

Arsphenamine and Neo-Arsphenamine—Voegtlin

$$\text{Therap. Index Arsphen.} \quad \frac{\text{M. L. D. (206)}}{\text{M. E. D. (9.9)}} = 20^1.$$

$$\text{Therap. Index Neo-Arsph.} \quad \frac{\text{M. L. D. (438)}}{\text{M. E. D. (20.6)}} = 24^1.$$

$$\text{Ratio} \quad \frac{\text{Therap. Index Neo-Arsphen.}}{\text{Therap. Index Arsphen.}} = 1.2.$$

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[6] Karsner and Hanzlick, *Jour. Pharm. and Exp. Therap.*, 1920, xiv, 479.

[7] Oliver and Yamada, *Jour. Pharm. and Exp. Therap.*, 1922, xix, 393.

$$\text{Ratio } \frac{\text{M. E. D. Arsphen.}}{\text{M. E. D. Neo-Arsph.}} = .48.$$

Arsphenamine and Gelatin-Arsphenamine

$$\text{Therap. Index Arsphen. } \frac{\text{M. L. D. (100)}}{\text{M. E. D. (10)}} = 10.$$

$$\text{Therap. Index Gel. Arsp. } \frac{\text{M. L. D. (140)}}{\text{M. E. D. (10)}} = 14.$$

$$\text{Ratio } \frac{\text{Therap. Index Gel. Arsp.}}{\text{Therap. Index Arsphen.}} = 1.4.$$

$$\text{Ratio } \frac{\text{M. E. D. Arsph.}}{\text{M. E. D. Gel. Arsph.}} = 1.$$

MINIMUM STERILIZING DOSE

Schamberg, Kolmer and Raiziss

$$\text{Therap. Index Arsphen. } \frac{\text{M. T. D. (105)}}{\text{M. S. D. (23)}} = 4.5.$$

$$\text{Therap. Index Neo-Arsph. } \frac{\text{M. T. D. (254)}}{\text{M. S. D. (40)}} = 6.3.$$

$$\text{Ratio } \frac{\text{Therap. Index Neo-Arsphen.}}{\text{Therap. Index Arsphen.}} = 1.39.$$

$$\text{Ratio } \frac{\text{M. S. D. Arsphen.}}{\text{M. S. D. Neo-Arsph.}} = .57.$$

$$\text{Therap. Index Arsphen. } \frac{\text{M. T. D. (90)}}{\text{M. S. D. (20)}} = 4.5.$$

$$\text{Therap. Index Gel. Arsph. } \frac{\text{M. T. D. (130)}}{\text{M. S. D. (20)}} = 6.5.$$

$$\text{Ratio } \frac{\text{Therap. Index Gel. Arsph.}}{\text{Therap. Index Arsph.}} = 1.4.$$

$$\text{Ratio } \frac{\text{M. S. D. Arsphen.}}{\text{M. S. D. Gel. Arsp.}} = 1.$$

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[1] The greater magnitude of these ratios as compared to our figures and those of Schamberg *et. al.* is the result of the high lethal dose. This is doubtless due to the shorter period of observation, 48 hours instead of two weeks, which Voegtlin used.

M. L. D. = minimum lethal dose.   M. S. D. = minimum sterilizing dose.
M. E. D. = minimum effective dose.   M. T. D. = maximum tolerated dose.

25 (1985)

Studies on anthrax infection.

By W. L. HOLMAN

[*From the Department of Bacteriology and Experimental Pathology, Stanford University, San Francisco, California*]

The unsettled problems of infection with *B. anthracis* are very many. Besredka has recently declared his belief that the cutaneous is the only route of infection. Intestinal anthrax is the commonest form in which the natural disease appears to be found. The question has been raised whether there is such an infection. The opposing view is that the findings and symptoms in the intestines are only part of a general infection and that the portal of entry is in the skin, usually about the mouth and nose, and the local reaction, if any, is overlooked. In carrying out some experiments to cover some of the doubtful points we undertook the feeding of guinea pigs, taking particular care not to contaminate the mouth cavity. The technic used by previous workers did not seem satisfactory so a simple method was tried which protected against these possible contaminations. Small gelatine capsules were filled with the culture to be used, the mouth was opened and the capsules placed in the back of the throat. The animal swallowed them promptly. A guinea pig was fed in this way with a virulent culture rich in spores and a daily culture of the feces was made in agar after heating to 75°C for 15 minutes. A fecal pellet was emulsified in 10 c.c. of saline and 1 c.c. was used for the plate. The colony characteristic of *B. anthracis* is easily recognized but isolations were made each day and tested on fresh animals. In this way we found virulent anthrax spores in the feces up to the seventh day. Two weeks after the feeding the animal was given a subcutaneous dose and died of anthrax. A second pig was fed in the same way and positive fecal cultures were obtained up to the fifth day. An accident resulted in this animal's death on the eighth day but no microscopic or cultural evidence of anthrax could be found. Four guinea pigs fed in the same way were studied up to the twenty-ninth day when they were killed by a subcutaneous route injection. These animals showed anthrax spores up to the tenth day

with negative intervals between. A number of other fed guinea pigs are still under observation. The results of these tests would indicate that the intestinal tract is not an easy portal of entry for *B. anthracis;* that healthy susceptible animals can carry virulent anthrax bacilli for a considerable time and by the distribution of the spores in their feces can be a ready means of spread.

In reading the literature it is evident that the portal of entry is frequently in doubt. In injecting rabbits subcutaneously in the ear we noted that the local reaction was often scarcely noticeable and that the characteristic edema only appeared in the cervical tissues some distance from the portal of entry and the amount was not always very pronounced. The explanation suggested itself that the firmer tissues of the ear did not allow the edematous fluid to collect at the point where the organism was establishing itself. In the fluid are numerous leucocytes and these may also be prevented from collecting in quantity. Is it not possible therefore to inject in such tissues a dose of anthrax spores too small to be fatal in the looser tissues but which can establish themselves where some of the defenses are held in check? To determine this point we took six guinea pigs and injected them with about 50 spores in 1/20 of a cubic centimeter of saline. Three were injected subcutaneously over the abdomen and three under the skin of the ear. The three former lived and two of the latter died, one in forty-seven hours, and one in eighty-four. It is to be noted that the guinea pig dying first had accidently received an extremely small dose. The point of the needle was seen to be through the ear when the injection was started and the needle was withdrawn at once and so, although undetermined, the amount in the wound must have been minute. The reason the needle was withdrawn was to prevent any undue irritation which would have interfered with the object of the experiment. The autopsy findings in both these animals would not have suggested where the injection had been made—a slight amount of edema on the side of the neck in one and a slight amount along the trachea in the other. These are suggestive findings and will need to be repeated many times.

ABSTRACTS OF COMMUNICATIONS, WESTERN NEW YORK
BRANCH.

Third meeting.

Geneva, New York, October 14, 1922

26 (1986)

The comparative fat content of the portal vein as determined by the presence of fat particles with the darkfield microscope.

By PIERRE A. FISH

[*From Cornell University, Ithaca, N. Y.*]

Although it is generally stated that not all of the fat ingested can be recovered from the thoracic duct, there is no very satisfactory evidence to show that fat is absorbed *directly* into the blood under normal conditions. Fat absorption through the villi into the lacteals and thoracic duct as the "molecular basis of chyle" and the entrance of these minute particles (chylomicrons) into the venous system has been known since the time of Boyle, Hewson and Gulliver, and is not difficult of demonstration.

The use of the darkfield microscope has been of distinct value in studying the increase and decrease of the chylomicrons during, and after, the period of fat absorption with the use of the ocular micrometer, following the method of Gage for counting these minute particles with approximate accuracy.

In connection with other study upon fat absorption, observations were made upon the chylomicron content of the blood of the aorta, jugular and portal veins at different periods of digestion with results as shown in the following table:

CHYLOMICRONS

Animal	Digestion		Aorta	Jugular V.	Portal V.
Kitten	6	hours	75	....	59
Kitten	4	hours	132	....	48
Kitten	7	hours	192	....	126
Cat	3½	hours	142	117	45
Kitten	6	hours	35	26	1
Kitten	7	hours	121	100	76
Cat	18½	hours	90	100	22
Dog	4	hours	13	15	5
Dog	24	hours	11	....	1.5
Cat	5½	hours	8	25.8	2
Cat	4	hours	100.2	152.2	33.4
Kitten (600 gms.)	4	hours	56.2	66.8	15

Dog	6	hours	241	215	237
Cat	5	hours	120	148	120
Cat	29½	hours	....	1	.8
Kitten (300 gms.)	1½	hours	117.2	130.6	119
Kitten (300 gms.)	4½	hours	111.6	116.8	122.8
Kitten (300 gms.)	5	hours	117.2	140.6	133.8
Kitten (300 gms.)	2	hours	55	78.4	87.4
Kitten (600 gms.)	19	hours	35.4	46.6	38

Following the course of the circulation, it should be noted that the liver has a double source of supply of these particles: (1) through the hepatic artery and (2) through the portal vein, the origin of which is principally from the capillaries of the mesenteric arteries in the villi of the intestine.

The result of the blood examinations was rather unexpected for it might naturally be assumed that the fat particles, distributed throughout the circulation, would be represented in the portal vein to the same extent as in the blood of other parts of the body, or if there were *direct* absorption into the blood even in greater numbers. In the majority of the cases the counts showed quite a distinct decrease in the number of the fat particles in the portal vein as compared with the jugular vein or aorta. Out of the total of 20 cases examined, 12 showed this decreased condition very clearly; the remaining 8 cases showed an approximately equal distribution of the fat particles in the blood of the three vessels. Six of the kittens in the list were unweaned and still being nursed by their mothers—four in one litter weighing approximately 300 grams each, and two in the second litter weighing approximately 600 grams each. The remaining fourteen included a few adults and a number of young animals which had passed beyond the nursing stage. Classifying the animals according to their degree of development the table shows that all of the nursing kittens, with one exception, possessed an approximately equal distribution of the fat particles in the blood of the three vessels. The exception was one of the second litter and although still nursing was able and did eat solid food.

In the more mature group only three showed the same condition as in the nursing group. One of this group might be considered as a neutral factor, since a period of 29½ hours had elapsed after the administration of fat and the chylomicron content of the blood would therefore be at a low ebb.

Another interesting difference was noted between the nursing kittens and the maturer animals. In some instances the red dye Sudan III was dissolved in the fat (butter) administered. In the nursing kitten the liver showed a slight pink color and when a portion of the liver was dried and extracted with ether a distinct pink color was shown in the extract. The same treatment applied to the liver of the more mature animal failed to give the pink color in every instance in which it was tried.

Two possible theories may be suggested to account for the diminution of the chylomicrons in the portal vein of the more mature animals: (1) that a certain proportion of them are eliminated in the intestinal capillaries and excreted into the contents of the intestine; (2) that some of them may be converted into a soluble form—through the absorption of bile salts—and can not therefore be observed under the microscope.

In the nursing kitten it would appear, from the chylomicron count and the Sudan III experiments, that the liver is utilized for the storage of fat, but in the more mature animals this function has apparently been diminished. The storage of fat in the liver of young animals was noted by Koelliker in 1856.

27 (1987)

Biological reactions of X-rays. Effect of X-rays on the rate of specific hemolysis.

By KARL F. CORI and G. W. PUCHER (by invitation)

[*From the State Institute for the Study of Malignant Disease and the Chemical Department of the Laboratories of the Buffalo General Hospital, Buffalo, New York*]

1. The radiation of the individual components of a hemolytic system does not increase the rate of hemolysis of that system.

2. Radiation of the whole hemolytic system increases the rate of hemolysis of that system.

3. X-rays influence the rate at which the equilibrium of a reaction is reached. Further studies are in progress to determine the effect of X-rays on the velocity of chemical reactions.

28 (1988)

Possibilities of increasing the value at depth of radiation from radium externally applied.

By WILHELM STENSTROM (by invitation)

[*From the State Institute for the Study of Malignant Diseases, Buffalo, New York*]

The methods that generally are used for external application of radium are rather crude and unsatisfactory. This refers especially to the old "radium pack" where a number of radium containers are spread out over a big surface, which is placed at a certain distance from the skin (usually 4 to 10 cm. away). The disadvantages are:

1. It takes a great distance and a long time to get a depth dose comparable to the depth dose from X-rays produced by 200 K. V.

2. The protection is unsatisfactory and the weight is troublesome for the patient.

3. The distribution of the radiation is poor and the total amount of radiation absorbed by the whole body is many times (say 10 times) the amount absorbed by a cylinder or parallelopiped of tissue directly under the pack.

The attempt to eliminate these bad features has resulted in an apparatus which gives very little radiation outside the cylinder directly under the pack. The amount absorbed inside this cylinder is at least one half of the total amount absorbed by the whole body.

The following table gives a comparison between the effectivity of the two packs. The filter is for both 2 mm. of brass and 1 mm. of aluminum.

	Distance from skin	Depth dose 10 cm. under skin	Erythema skin dose in:
Old pack	6 cm.	17 per cent.	6 gram hrs.
Old pack	20 cm.	34 per cent.	60 gram hrs. (approx.)
New pack	3 cm.	35 per cent. (approx.)	15 gram hrs. (approx.)

The new pack is attached to a modified X-ray stand and consists of three main parts.

(1) A cylindrical box of aluminum with lead-lined wall.

(2) A rotating lead-screen with holes of a complicated shape through which the rays are directed.

(3) A heavy lead cover.

29 (1989)

Influence of pancreatic perfusates upon the carbohydrate metabolism of depancreatized animals.

By HARRY D. CLOUGH, ARTHUR M. STOKES, C. B. F. GIBBS, NEIL C.

STONE and JOHN R. MURLIN.

[*From the Physiological Laboratory of the University of Rochester, Rochester, N. Y.*]

Following the method of perfusion introduced by Clark[1], the effect of pancreatic perfusates upon the carbohydrate metabolism of entire animals was studied. The respiratory metabolism, the blood sugar, and the D:N ratio were studied in cats and dogs after depancreatization.

Perfusates were made by perfusing aseptically and at body temperature the pancreases of cats, dogs, pigs, and oxen with Locke's solution. The first perfusates were made with an alkaline medium, later perfusates were made with Locke's solution modified by the substitution of HCl varying in strength from 0.1 per cent. to 0.7 per cent. for sodium bicarbonate.

Respiratory quotients were obtained by a modified Jaquet method, the animal being confined in a respiration chamber through which a continuous stream of air was drawn for ventilation and from which a continuous sample of air was removed for analysis. In a later modification the stream of air from the respiration chamber was directed into a large gasometer for a twenty-minute period and the samples for analysis withdrawn from this total volume. Analyses were made by the Henderson and Bailey modification of Haldane's apparatus. The respiration chamber was frequently checked by analyses of outside air and by burning alcohol in it.

D:N ratios and respiratory quotients typical for diabetes were obtained in the depancreatized animals.

Following the administration of sugar in the form of dextrose or sucrose by stomach tube pancreatic perfusates were given subcutaneously, intravenously, and intraperitoneally. Significant increases were shown in the respiratory quotients and marked

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1 Clark, Admont H., *Jour. Exp. Med.*, 1916, xxiv., 621.
Ibid, 1917, xxvi, 721.

drops were observed in the blood sugar level (frequently to points below normal) and marked changes were demonstrated in the D:N ratio. These changes were demonstrated both for alkaline and for acid perfusates, but were greater and more constant with acid perfusates given neutral.

30 (1990)

The influence of pancreatic extracts upon the carbohydrate metabolism of depancreatized dogs.

By C. B. F. GIBBS, HARRY D. CLOUGH, NEIL C. STONE and JOHN R. MURLIN.

[*From the Physiological Laboratory of the University of Rochester, Rochester, N. Y.*]

The experimental work reported dealt with the effects of extracts prepared in various ways from the pancreas of the dog, pig and ox upon the blood sugar, D:N ratio, respiratory quotient and clinical condition of depancreatized dogs.

A dog was given two days to recover from the operation and to become totally diabetic as shown by a D:N ratio of 2.8 or more. The dogs were fed ground beef in increasing amounts after the first day. The extracts were administered by stomach tube, intramuscularly, intravenously, subcutaneously and intraperitoneally.

Extract given by stomach tube caused no or very little fall in blood or urinary sugar. Injections of the extract into vein, tissue and peritoneum have been followed by abrupt lowering of the sugar in the blood and urine, the former at times even below normal and the latter has been made to completely disappear even when glucose had been previously administered. In the majority of cases the lowest level of blood sugar was reached in 4 hours from the time of injection whether the extract was given into vein, peritoneum, muscle or under the skin. Since harmful effects were experienced more frequently when the extracts were injected intravenously, intramuscularly and intraperitoneally than when given subcutaneously the last method was used most.

The first preparations used in this series of experiments were extracts of freshly removed, macerated dog's pancreas given

without further treatment. These proved exceedingly toxic causing extensive sloughing and ulceration of the tissues into which they were injected, and sometimes even caused the death of the dog. These wounds were sterile unless secondarily infected. Simple alcoholic extracts likewise produced toxic effects, particularly when only small volumes of alcohol were used and the trypsin was for this reason not wholly destroyed. Later these toxic effects were very largely eliminated by purification of the extracts.

Tables and graphs of experimental data were given showing abrupt falls in blood sugar, disappearance of urinary sugar and rise of the respiratory quotient following injection of extracts.

31 (1991)

Three months study of the influence of the anti-diabetic substance on a case of severe diabetes.

By C. CLYDE SUTTER and JOHN R. MURLIN.

[*From the Physiological Laboratory of the University of Rochester, Rochester, N. Y.*]

Anti-diabetic substance, prepared at the University of Rochester was given to a case of severe diabetes mellitus (adult) by the duodenal tube, by mouth and by subcutaneous injections. Accurate study was made to determine the potency of the pancreatic extract, the dosage required and the most efficient and practical method of administration.

Glycosuria had been present at least for five years and was progressively increasing. Acidosis was constant during the past year. At the time of beginning this study the elimination of urinary sugar was greater than the intake of carbohydrate.

The patient was placed in the Rochester General Hospital, July 6, 1922, and studied while on a weighed diet consisting of carbohydrate 29.5 gms., protein 69 gms., fat 107.48 gms., and total calories 1394. During this study the blood sugar averaged from 0.410 to 0.513; the urinary sugar averaged from 32 to 45 grams; and the acetone and diacetic acid was 2+.

Anti-diabetic extract was given fifteen times through the duodenal tube from July 12, 1922 to July 29, 1922 inclusive. The blood sugar was reduced, reaching its lowest, 0.241 on July 26,

1922. The urinary sugar at this time reached its lowest point, 9.17 gms. Acetone was reduced about one half the former quantity and diacetic acid was absent on fourteen of the eighteen days treatment.

The patient was transferred to his home July 31, 1922 and kept at rest and on a diet consisting of carbohydrate 28.52 gms., protein 76.16, fat 127.04, and total calories 1,900. No treatment was given from July 29, 1922 to August 13, 1922. During this time the blood sugar rose somewhat but did not return to the level before treatment was started. The urinary sugar averaged from 39 to 52 grams. The acetone and diacetic acid 1+.

Forty-five injections were given subcutaneously during the period from August 13, 1922 to October 13, 1922. From one to three injections were given daily with occasional intermission. Extracts secured by different methods were used. By means of the subcutaneous method of injection, the blood and urine sugar, acetone and diacetic acid were reduced to normal. The clinical symptoms entirely disappeared. Marked reduction in blood sugar was at times noted. On August 25, 1922 the blood sugar was reduced from 0.320 to 0.200 six hours after a single injection of the pancreatic extract. On September 9, 1922 the blood sugar was reduced from 0.200 to 0.092, a reduction from 0.328 to 0.092 in twenty-three hours by four injections subcutaneously. The blood sugar rose over night to 0.200. In six hours by a single injection it was reduced to 0.070. The urinary sugar, acetone and diacetic acid disappeared entirely. The diet was increased to carbohydrate 50.52 gms., protein 119, fat 167, and total calories 2,500. The urinary sugar, acetone and diacetic acid remained absent. The blood sugar showed a slight rise.

Some of the earlier injections showed some pain, local edema and occasionally temporary redness. Only three of the forty-five injections showed definite local reaction. Later injections were non-toxic and non-irritating. Pain is no greater than can be accounted for by tissue distention.

Subcutaneous injections were discontinued October 4, 1922 and the extract given by mouth for two days. During the next three days the extract was given in salol-coated capsules. Neither method was plainly effective.

Subcutaneous injections were again given and the blood and urinary sugar were reduced.

32 (1992)

Properties and methods of preparation of the anti-diabetic substance (glucopyron) generated by the pancreas.

By JOHN R. MURLIN.

[*From the Physiological Laboratory of the University of Rochester, Rochester, N. Y.*]

Repetition of several older methods of extraction of pancreas proved that with very slight modification any one of them was adequate to demonstrate the presence of the anti-diabetic substance. The essential steps in its preparation are: (1) destruction of trypsin; (2) precipitation of extraneous proteins; (3) concentration; and (4) removal of irritant substances. Banting[1] and Macleod[2] appear to have adopted exclusively the method of alcoholic extraction and have given the name *insulin* to the alcoholic extract. Just as potent and non-toxic extracts may be prepared with aqueous media. The active substance is *not* all in the final precipitate with absolute alcohol as stated by Collip[3]. It is non-dialyzable through vegetable parchment in four hours' immersion in running water; it is not precipitated by most of the ordinary reagents employed for the precipitation of proteins. It withstands boiling for 5 minutes in acid (N/10) media; and it may be adsorbed on several different reagents commonly used for this purpose.

Proof that an aqueous extract given with small amount of alkali (N/20 NaOH) by stomach tube will cause oxidation of sugar in the depancreatized dog was given by Kramer[4] and the writer in 1916. For the active substance itself (not the entire alcoholic extract) however obtained and whatever its chemical nature the name *Glucopyron* (*Glykos,* sugar and *Pyron,* burning) is suggested.

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[1] Banting and Best, *Journ. of Lab. and Clin. Med.*, 1922, vii, 464.

[2] Macleod *et al.*, *Amer. Journ. of Physiol.*, 1922, lxii, 162.

[3] Collip, J. B., *Trans. Roy. Soc. of Canada*, 1922, xvi.

[4] Murlin and Kramer, *Journ. of Biol. Chem.*, 1916, xxvii, 516.

SCIENTIFIC PROCEEDINGS

33 (1993)

A modified Gram stain.

By NICHOLAS KOPELOFF and PHILIP BEERMAN.

[*From the Department of Bacteriology, Psychiatric Institute,
Ward's Island, New York City.*]

The following method of Gram staining is based upon the satisfactory results obtained by the use of modifications devised by Burke and Atkins:

1.   Air dry a thinly spread film and fix with least amount of heat necessary to kill the organisms and fix them to the slide.

2.   Flood slide with dye solution. This is prepared by previously mixing in a beaker about 30 drops of a 1 per cent. *aqueous* solution of methyl violet 6B (Coleman and Bell) with 8 drops of a 5 per cent. solution of sodium bicarbonate. Allow the mixture to remain on slide 5 minutes or more.

3.   Flush off the excess stain with the iodine solution and cover with fresh iodine solution for 2 minutes or longer. The iodine solution consists of 2 gm. iodine dissolved in 10 c.c. normal sodium hydroxide solution, to which is then added 90 c.c. of water.

4.   Drain off the excess iodine solution, without blotting (no water being used) but the film is not permitted to become dry.

5.   Add acetone (100 per cent.) drop by drop until no color is seen in the drippings from the slide, which is slightly tilted. This usually requires less than 10 seconds, and should be reduced to a minimum.

6.   Air dry the slide.

7.   Counter stain for 10-30 seconds with 0.1 per cent. aqueous solution of basic fuchsin.

8.   Wash off excess stain by short exposure to tap water and air dry.   If slide is not clear, immersion in xylol is recommended.

This method has yielded particularly good results in staining milk slides for *Bacillus Acidophilus* and in staining fecal specimens.   By this method gonococci and diphtheria bacilli are particularly well differentiated and more easily identified than by the older methods.   The same was found to be true for a number of common pathogens and saprophytes studied.

34 (1994)

Permeability of the cell: the surface as contrasted with the interior.

By ROBERT CHAMBERS.

[*From the Department of Anatomy, Cornell University Medical College, New York City.*]

Protoplasm is known to be permeable to some substances and not to others.   The microinjection method appears to be the only method of determining whether this semi-permeability is a property of the entire mass of protoplasm or of its surface film only.

Kite[1] injected cells by the Barber pipette method[2] and claimed to have proved that semi-permeability is a property of all portions of protoplasm. His conclusions are open to criticism owing to the extreme difficulty of the method and to his having overlooked the extraordinary ability of protoplasm to form surface films over cut surfaces. The results which I[3] obtained are directly opposed to Kite's conclusions. Recently I have devised[4] a micro injection apparatus with which one can, with remarkable ease and accuracy, inject living cells by means of pipettes with a bore less than one micron in diameter.

A half molecular ammonium chloride solution in sea water is acid to neutral red. Starfish eggs stained with neutral red and immersed in this acid solution turn yellow, owing to the penetration of only the alkaline group of the dissociated salt. If, however, stained eggs be placed in an alkaline sodium bicarbonate solution, they give evidence of the penetration of only the carbonic acid group. These findings are being reported by Jacobs in the *Journal of General Physiology*. They confirm the observations of previous investigators that weak acids and bases freely penetrate living cells whereas strong acids and bases do not.

My experiments, described in a forthcoming number of the *Journal of General Physiology*, consisted in the injection of NH_4Cl and $NaHCO_3$ into starfish eggs stained with neutral red. In the case where NH_4Cl was used the injected area immediately changed to a red color and then underwent cytolysis. The color change and accompanying cytolysis spread from this area till it reached the cortex of the egg which disintegrated from within outward. In some cases this spread was arrested by the formation of a surface film which converted the injected and disintegrated area into a vacuole. This experiment demonstrates that $\frac{1}{2}$ M NH_4Cl, which causes an alkaline color change within eggs when its effect is transmitted only through the surface film, will, when injected into the interior of the eggs, produce

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[1] *Amer. Jour. Physiol.*, 1915, xxxvii, 282.

[2] Philipp, *Jour. Sc.*, Sec. B., Trop. Med., 1914, ix, 307.

[3] *Jour. Pharmacol. Exp. Therap.*, 1919, xiv, 75; PROC. SOC. EXP. BIOL. AND MED., 1920, xviii, 66.

[4] *Anat. Rec.*, 1922, xxxiv, 1.

the acid color change and accompanying cytolysis characteristic of free HCl.

When $NaHCO_3$ was introduced into a stained egg the injected area immediately turned yellow and cytolysis with liquifaction took place. The change to a yellow color and accompanying cytolysis spread throughout the cell. This showed that NaOH, which can not penetrate the surface film, will exert its characteristic effects if introduced directly into the interior of the cell.

The semi-permeability of a living cell is a function of its surface film. It is immaterial whether this film be that of the original cortex, a film newly formed over a cut surface, or a film that surrounds an artificially induced vacuole within the cell. As long as a surface film exists, neither the acid group of the NH_4Cl nor the alkaline group of the $NaHCO_3$ can penetrate protoplasm. On the other hand, if injected beneath the surface film they freely permeate the protoplasm.

35 (1995)

Fat transport in the body—changes in the lipoid content of the blood and lymph during fat absorption in the dog.

By H. C. ECKSTEIN (by invitation).

[*From the Sheffield Laboratory of Physiological Chemistry, Yale University, New Haven, Conn.*]

After alimentary absorption of fat the content of both the total fatty acids and the phosphatides of the blood is increased. According to Bloor the phosphatides are synthesized from neutral fats by the blood corpuscles. It is also conceivable that phosphatide synthesis occurs during the passage of the fat components through the intestinal wall. To test this hypothesis the thoracic lymph and blood collected before and after introduction of olive oil into the duodenum of dogs previously starved for 18 hours was analyzed as follows:

DOG, 9 KILOS IN BODY WEIGHT—A TYPICAL EXPERIMENT
(The analyses are expressed in milligrams per 100 c.c. of blood or lymph)

Period before or after fat injection	Lymph			Blood	
	Total fatty acids	Phos-pha-tides	Volume of lymph	Total fatty acids	Phos-pha-tides
Before	280	160	11 c.c.	600	440
0-2 hours after............	270	180	39 c.c.	570	430
2-4 hours after............	480	210	19 c.c.	660	550
4-6 hours after............	1,550	240	45 c.c.	630	500
6-8 hours after............	1,670	230	8 c.c.	810	490
8-10 hours after............	1,600	200	47 c.c.	800	510
10-12 hours after............	1,700	200	20 c.c.	800	550
12-14 hours after............	2,400	200	11 c.c.	790	520
14-15 hours after............	1,720	200	14 c.c.	560	490

The results show clearly that while the total fatty acids of the lymph increase rapidly during fat absorption, the content of phosphatides remains unchanged. Hence there is no reason to believe that synthesis of phosphatides takes place before the fat leaves the thoracic duct.

36 (1996)

The inorganic phosphorus and calcium in maternal and foetal blood.

By ALFRED F. HESS and M. MATZNER.

[*From the Department of Pathology, College of Physicians and Surgeons, Columbia University, New York City.*]

This study included examinations of the blood of pregnant women in the latter months of pregnancy, of the cord blood of infants, and of mothers' blood within forty-eight hours after labor. The calcium was determined on serum by the Lyman method,

and the inorganic phosphorus on the whole blood, using the Bell and Doisy method, without the addition of oxalate or other anticoagulant. The calcium determinations did not indicate significant variations from the normal; the eleven specimens taken antepartem gave an average of 10.4 mg. per cent., whereas eighteen taken postpartem averaged 9.75 mg. per cent. It is possible that tests taken at various periods of pregnancy would give more significant figures. The calcium of the cord blood in eighteen cases gave an average of 10.75 mg. per cent. Howland and Marriott[1] found a normal percentage of calcium in three tests of cord blood. Jones and Nye[2] have reported a calcium content averaging 12.6 mg. per cent. in the plasma of five babies under the age of 14 hours.

As far as we know there have been no tests for phosphorus in the cord blood of infants. McKellips, De Young and Bloor[3] tested the blood of infants during the first 26 days of life and found the inorganic phosphorus the same in the plasma of infants and of adults, and that in the corpuscles somewhat lower in infants. The average inorganic phosphorus of twenty-one cases we found to be 3.71 mg. per cent.; the mothers' blood in these cases averaged 2.89 mg. when taken a day or two following labor. In twelve cases where the inorganic phosphorus was tested during pregnancy, the percentage was 2.77 mg. There are two comments suggested by these figures. First, that inorganic phosphate is considerably higher in the blood of the foetus than in that of its mother. Second, that although the content of inorganic phosphate is higher in the foetal than in the maternal blood, it is markedly lower than that of the young infant. The normal content of inorganic phosphorus in infants is about 4.5 mg. when tested by this method. The percentage 3.71 mg. which we obtained is generally indicative of rickets in postnatal life. Radiographs of the epiphyses showed no evidence of rickets in these cases.

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[1] Howland, J. and Marriott, W. McK., *Proc. Amer. Ped. Soc.*, 1916, xxviii, 202.

[2] Jones, M. R. and Nye, L. L., *Jour. Biol. Chem.*, 1921, xlvii, 321.

[3] McKellips, G. M., De Young, I. M. and Bloor, W. R., *Jour. Biol. Chem.*, 1921, xlvii, 53.

37 (1997)

Some limiting factors in the use of picramate as a measure of reduction.

By SISTER MARY DENISE, S. S. N. D., and A. R. ROSE.

[*From the Department of Chemistry, Fordham University, New York City.*]

This report pertains to the colored solutions formed by the reduction of picric acid. The color is not due to a single substance but to a mixture of several colored substances. In solutions where the color is due to some one substance, there is a simple and definite ratio between the concentrations and the color values. When the color is due to two or more substances a definite ratio holds for differences in concentrations only within relatively narrow limits. The colorimetric determination of picrate reduction mixtures is therefore limited. It is thought that this limitation can in part be counteracted by isolating the most characteristic component of the color. This is done by extracting the products after the reduction of picrates with immiscible organic solvents, shaking this extract with an alkaline solution. The alkaline solution assumes a color which is qualitatively very like the original reduction mixture. The number of colored constituents has been reduced. The color values of the reduction mixture and the isolated color component are affected by change in temperature. One degree centigrade decreases the color value one per cent. Salts, cane sugar and alkalies increase the color value. These tests were made by diluting picramate mixtures with molar solutions and comparing them with a standard prepared in the same manner as the picramate mixtures which were tested. In half-molar solutions there is but a slight error for sodium chlorid; for the sugar it came to 10 per cent., and for potassium carbonate to 12 per cent. Molar solutions gave an error of 17 per cent. in the case of salt, 14.5 per cent. for sugar, and 16 per cent. for potassium carbonate. Sodium hydroxide at this concentration caused an error of 8 per cent. Higher concentrations made very difficult readings. Sodium chlorid in three-molar concentrations decreased the readings 25 per cent. and five-molar 29 per cent. Sodium hydroxide in two-molar solution caused a 25 per cent. decrease

and four-molar a 41 per cent. In all these cases there was an increase in color value. This is probably due to an increase in a brownish color component. There was some precipitation. In many instances there was a finely divided sediment in the picrate reduction mixture. For this reason a study was made as to the effect of filtration. Ashless paper was used and in all but very few cases filtration was followed by a decreased color value. The error from such a cause ranged from 0.5 to 6 per cent. A solution passed through six 9-cm. papers had a decrease in color value amounting to 17 per cent.

38 (1998)

Factors involved in the quantitative reduction of the tissues in the stomach and intestine in amphibian larvæ during metamorphosis.

By ALBERT KUNTZ.

[*From the Department of Anatomy, St. Louis University School of Medicine, St. Louis, Mo.*]

According to data recorded elsewhere[1], the following changes in the digestive tube occur in Rana pipiens and Ambystoma tigrinum during metamorphosis.

	R. pipiens	*A. tigrinum*
Average reduction in length of stomach and intestine	82.5 per cent.	45.8 per cent.
Average reduction in weight of tissue in stomach and intestine	56.5 per cent.	46.9 per cent.

The rôle of autolysis and phagocytosis in the quantitative reduction of the tissues in the gills, fins, and tail of amphibian larvæ during metamorphosis has been studied repeatedly. Autolysis and phagocytosis also account for a large part of the quantitative reduction in the tissues in the stomach and intestine. The extrusion of tissue elements, especially from the mucosa and submucosa, into the lumen of the stomach and intestine

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[1] Kuntz, A., Anatomical and physiological changes in the digestive system during metamorphosis in Rana pipiens and Ambystoma tigrinum. *Journal of Morphology* (not yet published).

is an additional factor which the writer has not found recorded hitherto.

As the stomach and intestine undergo reduction in length, their walls become thicker. As pointed out by Ratner[2], the increase in the thickness of the several layers in the walls of these organs is brought about not by active cell proliferation but by rearrangement and aggregation of the elements already present. During the early phases of this process many of the smaller blood vessels become obliterated or constricted; consequently autolysis of many of the tissue elements is initiated. The tissues become infiltrated with leucocytes and phagocytosis, doubtless, plays a part in the removal of tissue fragments.

During the progress of metamorphosis, the stomach and intestine become free from ingested material and masses of cellular debris, including nuclei in which a portion of the chromatin still reacts to the basic stain, occur in the lumen throughout the stomach and small intestine. The gastric and intestinal epithelium becomes increasingly irregular as metamorphic changes advance. The nuclei of all the epithelial cells no longer remain at approximately the same level, but many of them approach the free surface of the epithelium. Not infrequently the nuclei of epithelial cells protrude and the cells slough into the lumen. Both in the stomach and intestine, cells from the mucosa, including many of the invading leucocytes, also slough into the lumen. In some instances definite points may be observed at which the epithelial cells have separated to permit the extrusion of aggregates of cells from the deeper layers of the mucosa and the submucosa; in others, only sufficient separation of epithelial cells occurs to permit the displacement of individual cells from the deeper layers into the lumen. The frequency with which the extrusion of cells either singly or in aggregates from and through the epithelium occurs and the volume of the cellular debris present in the lumen of the stomach and intestine suggest that a large part of the quantitative reduction of the tissues in these organs is brought about by this process.

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2 Ratner, T., Metamorphose des Darmes bei der Froschlarve, Dorpat, 1891.

39 (1999)

The effect of ultra-violet rays on rats, deprived of vitamine A in their diet.

By OLIVE SHEETS and CASIMIR FUNK.

[*From the Biochemical Laboratory, College of Physicians and Surgeons, Columbia University, New York City.*]

Experiments have demonstrated that both xerophthalmia and rickets can be prevented and cured by the administration of cod liver oil, and that rickets can be cured and prevented by sunlight and ultra-violet light.

We know that the absence of vitamine A from the diet will produce xerophthalmia, and it was believed by some workers, notably Mellanby, that the same deficiency is responsible for rickets. It is generally agreed, however, that the two above named pathological conditions are distinct entities and our experiments bear out this opinion. We have investigated whether light has any noticeable effect on growth and xerophthalmia in analogy to its effect on rickets, presumably on account of a better utilization of the stored vitamine A in the body.

With this view in mind the following experiment was undertaken:

Twelve young rats of the same age were placed upon a diet as nearly free as possible from vitamine A. The diet used was that of Osborne and Mendel (with oxidised casein and oxidised lard), and as source of B vitamine, a yeast preparation was added. The rats were divided into two lots of six each. One lot was exposed for five minutes daily to the action of a carbon arc lamp for three weeks, and for the remainder of the experiment for three minutes daily, except Sunday, to ultra violet light. The animals were kept in an animal room that received the usual amount of daylight.

CONTROLS

Rat. No.		Init. weight February 2	Fin. weight June 17	Xerophthalmia after
25	♂	36 grams	70	134 days
26	♂	28 grams	69	129 days
29	♂	37 grams	86	137 days
32	♀	43 grams	92	....
33	♀	33 grams	74	....
35	♀	51 grams	102	....

RAYED ANIMALS

Rat. No.	Init. weight February 2	Fin. weight June 17	Xerophthalmia after
27 ♂	35 grams	72	128 days
28 ♂	29 grams	70	128 days
30 ♂	32 grams	80	....
31 ♀	40 grams	88	....
34 ♀	36 grams	80	140 days
36 ♀	59 grams	110	....

It can be seen from the table that there was no difference as regard the rate of growth and incidence of xerophthalmia as the result of treatment with ultra violet light. We could not tell whether the earlier development of xerophthalmia in the males is of any significance or only purely accidental. A histological examination of the ribs of both series of rats was undertaken with the view to detecting rickets. The results were negative.

We are indebted to Dr. Hess for placing at our disposal the sources of light mentioned. We also thank the pathological department of the College of Physicians and Surgeons for help extended to us.

40 (2000)

The influence of light and darkness upon the development of xerophthalmia in the rat.

By G. F. POWERS, E. A. PARK and NINA SIMMONDS.

[*From the Department of Pediatrics, Yale University School of Medicine, New Haven, Connecticut, and the Department of Chemical Hygiene, Johns Hopkins University, Baltimore, Maryland.*]

The preventive influence of direct sunlight and of radiation with the mercury vapor quartz lamp upon the development of experimental rickets in rats has been demonstrated in experiments reported in previous studies. A logical further step was to determine whether or not direct sunlight and radiation with the mercury vapor quartz lamp would also prevent the development of xerophthalmia in rats fed diets which, under ordinary

conditions of roomlight, would lead to the development of both rickets and xerophthalmia and of xerophthalmia alone. If this information could be ascertained it would be a valuable contribution to the study of xerophthalmia and by analogy would suggest either the unity or the divisibility of the factors contained in cod liver oil, which prevent and cure both xerophthalmia and rickets. It was desired also to determine whether or not different combinations or groups of light rays—as for example, direct sunlight, quartz lamp radiations and roomlight—have the same or similar influence upon rats fed xerophthalmia producing diets. It was conceivable also that complete absence of light rays might have a different effect upon experimental animals than that produced by radiations showing either a complete solar spectrum (direct sunlight) or spectra considerably different from sunlight (quartz lamp radiation and roomlight).

On October 22, 1921, fifteen young albino rats were placed on a diet low in fat-soluble A and phosphorus (3127). Previous experience had shown that young rats on this diet would develop rickets and xerophthalmia. The animals were divided into three groups of five each. One group was to be kept in a laboratory room screened with ordinary window glass; a second group was to be kept in total darkness, excepting for the rays from a red electric light bulb such as is used in photographic dark rooms[1]; the third group was to be kept in ordinary roomlight, but was to be radiated with a mercury vapor quartz lamp. On November 15, 1921 another experiment was started using twenty-six young albino rats. These animals were placed on a diet low in fat-soluble A and poor in proteins of good quality (3392). Previous experiments had shown that this diet produces xerophthalmia in young rats. These animals were divided into groups and subjected to the same experimental conditions as detailed above. In these preliminary experiments it was found that all of the animals failed to grow, became emaciated and developed xerophthalmia. On the doubly deficient diet only the rats treated with the violet rays failed to develop rickets.

It was decided to repeat these preliminary experiments under conditions more favorable for study, and in addition to expose rats to the effect of direct sunlight. Accordingly, on April

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[1] This bulb was lighted only when the animals were being fed or inspected.

1, 1922, twelve young rats were placed on the doubly deficient diet (3127); they were divided into four groups (each animal in a separate cage). The first three groups were subjected to the same conditions of light and darkness as heretofore described in the report of the preliminary experiments; the fourth group was exposed to direct sunlight. The daily period of exposure to sunlight averaged four hours and to ultra violet radiation thirty minutes. On April 20, 1922 a parallel experiment was begun, using eighteen young rats and the diet producing xerophthalmia only (3392).

Diet 3392 because of the inadequacy of its proteins both in quantity and quality produces xerophthalmia in rats more quickly than the doubly deficient diet 3127.

The results of these experiments and the conclusions which they warrant may be summarized as follows:

On both diets the rats in the roomlight, darkness and violet lamp groups developed xerophthalmia early, ceased to grow, became markedly emaciated and died. On the doubly deficient diet (3127) only the animals subjected to violet lamp radiation and sunlight exposure failed to develop rickets.

Radiation with the mercury vapor quartz lamp for thirty to sixty minutes daily does not prevent the development of xerophthalmia but promotes bodily vigor to a very limited degree.

The rats exposed to direct sunlight (with one exception) never developed xerophthalmia until the animals in the other groups had developed the disease and died. In some of these "sunlight" rats xerophthalmia developed late and the animals died; in others the disease was manifested late also but in recurring mild attacks; in still others it occurred in a mild form from which there was spontaneous recovery. Two of the rats never developed xerophthalmia at all; one of these animals and one in which there was spontaneous recovery were still living and free from xerophthalmia when the experiment was terminated, five months after it was begun. None of the animals had rickets.

In the "sunlight" animals which developed xerophthalmia and in all the rats in the other groups the disease was more severe in the animals on diet 3392.

The rats exposed to sunlight made an initial gain in weight which in the animals on the rickets-xerophthalmia producing diet

(3127) was maintained and in those on the diet producing xerophthalmia alone (3392) never entirely lost.

Exposure to direct sunlight protects rats from xerophthalmia to a limited degree, dependent in part at least on the extent of the dietary deficiency in fat-soluble A and in other factors, particularly proteins of good quality and upon the duration and constancy of the exposure. In some animals the disease develops regardless of sunlight; in others it is on the surface or just submerged; while in a few it never develops at all.

In this paper when the expression "exposure to direct sunlight" is used, it is inclusive of all that is ordinarily indissolubly associated with sunlight exposures. Sunlight itself, the effect of changes in the atmosphere by solar radiation, temperature, out-of-door air, these and other factors unknown to us acting singly or in combination may be responsible for the results usually attributed simply to "sunlight."[2] These experiments bring out above all else that exposure to direct sunlight and out-of-door air promotes in a very wonderful way the health, bodily vigor and longevity of animals which otherwise are unable to adapt themselves to markedly adverse environmental conditions.

Sunlight probably exerts no specific anti-xerophthalmic influence but acts by raising the level of the cellular activity of the organism to a point where the progress of the disease is held in check or allowed to advance very slowly and with relatively little disturbance.

Previous experiments have shown that sunlight contains an equivalent of the unknown factor in cod liver oil which promotes the normal formation of bone and in that sense may be spoken of as anti-rachitic. The experiments herein reported indicate that sunlight does not contain at all or only to a very slight degree the equivalent of the anti-xerophthalmic factor in cod liver oil—fat-soluble A.

By analogy, therefore, these experiments suggest that there are in cod liver oil two distinct factors—one preventive and curative of rickets, and the other preventive and curative of xerophthalmia. Sunlight can compensate for the absence of the one but not completely of the other.

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[2] Experiments are now in progress which seem to indicate that out-of-door air does have a favorable influence upon rats fed a rickets-producing diet.

41 (2001)

The pancreatic factor in intestinal obstruction.

By H. B. EISBERG.

[*From the Department of Experimental Surgery, University and Bellevue Hospital Medical College, New York City.*]

In a previous communication[1] some experiments on the pancreas in relation to intestinal obstruction were reported. This presentation includes a duplication of the work with additional data. Twenty-three dogs were used. The experiments were divided into five groups.

Group I: Duodenal occlusion, pancreatic occlusion,[2] number of dogs, 5.

Average duration of life, 76.6 hours. Observations: A mild toxæmia developed which did not stimulate the toxæmia of duodenal obstruction. Autopsy revealed a slight congestion of the duodenal and colon mucosa. Microscopic examination of the pancreas[3] showed a capillary congestion, nuclei indistinct and the ducts filled with secretion.

Note: An accidental traumatic pancreatitis and a partial devitalization of the oral duodenum was responsible for a severe toxæmia in two of these cases. These variables were not included in estimating the mean duration of life.

Group II: Duodenal occlusion, partial pancreatic resection (duct bearing portion), number of dogs, 3.

Average duration of life, 43.3 hours. Observations: The symptoms of duodenal obstruction were greatly accentuated owing to the associated pancreatitis. Autopsy revealed a congestion of the peritoneum, also a slight congestion of the duodenal and colon mucosa. Fat necrosis was present in each case. Microscopic examination of the pancreas showed a hæmorrhagic infiltration with cells in different degrees of auto-digestion.

Group III: Duodenal occlusion, pancreatic excision, number of dogs, 5.

1 Eisberg, H. B., *Annals of Surgery*, 1921, lxxiv, 584.

2 Sweet, J. E., Peet, Max M., Hendrix, B. M., *Annals of Surgery*, 1916, lxiii, 720.

3 Fraser, A., Personal communication.

Average duration of life, 70.3 hours. Observation: The severity of the toxæmia of duodenal obstruction is greatly diminished when the pancreas is excised. Autopsy revealed a slight congestion of the mucosa of the duodenum and terminal colon.

Note: A severe toxæmia occurred in two cases; the result of general peritonitis in the one and partial devitalization of the duodenum in the other. These variables were not included in estimating the mean duration of life.

Group IV: Duodenal occlusion, pancreatic excision, pancreatic transplant (auto), number of dogs, 4.

Average duration of life, 29.7 hours. Observation: A severe toxæmia developed within a few hours, the result of devitalized tissue produced by the digestion of the bed of the pancreatic transplant. Autopsy revealed congestion of the peritoneum duodenal and colon mucosa from a slight to a moderate degree.

Group V: Ileal segmental exclusion; bilateral occlusion; reconstruction of intestinal canal; pancreatic tissue (homo) placed within isolated segment[1][2]; number of dogs, 6.

Average duration of life, 35.7 hours. Observations: A severe fulminating toxænia developed within 18 hours. Autopsy revealed a devitalized segment. A rupture of the segment was found in three animals with resulting fat necrosis and peritonitis.

Note: In one animal the segment remained viable. The resulting toxæmia stimulated duodenal occlusion. The duration of life was 72 hours. This variable was not included in estimating the mean duration of life.

42 (2002)

A new method of testing liver function with phenoltetrachlorphthalein. III. Clinical report.

By SANFORD M. ROSENTHAL (by invitation).

[From the Department of Pharmacology, Johns Hopkins University, Baltimore, Maryland.]

The author's method consists of determining the rate of disappearance from the blood stream of phenoltetrachlorphthalein,

after the intravenous injection of 5 mg. per kilo. A simplified method of determining the amount of dye in the plasma has been devised: a series of standards for comparison is prepared by adding varying amounts of the dye to plasma prior to the injection.

The test was performed upon ten normal individuals and ten control cases of extra hepatic disease. Seventeen cases of liver disease of various types were tested and results fully bore out experimental findings; striking degrees of retention of the dye in the blood were present where known damage to the liver existed. Results are quantitative, they have harmonized with the clinical evidence of the extent of liver damage and it is believed that they give an index of the functional capacity of the entire liver.

43 (2003)

The effect of sunlight upon the concentration of calcium and of inorganic phosphorus of the serum of rachitic children.

By BENJAMIN KRAMER and FRANK H. BOONE.

[*From the Department of Pediatrics, Johns Hopkins University, Baltimore, Maryland.*]

Recent studies have shown that the bone lesions of rickets may be healed by a variety of measures (administration of a proper diet, or of cod liver oil, radiation with mercury vapor quartz lamp, carbon arc lamp, cadmium open spark, etc.) Hess and Unger[1] claim to have obtained a similar result by exposing children suffering with rickets to direct sunlight.

Howland and Kramer[2] have demonstrated that with active rickets, unassociated with tetany, there occurs regularly a marked reduction of the concentration of inorganic phosphorus in the serum. In some instances there was also a moderate reduction of the calcium concentration. With active rickets the reduction is such that when the concentration of calcium expressed in mg. per 100 c.c. of serum is multiplied by that of inorganic phosphorus similarly expressed, the product does not

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1 Hess, A. F., and Unger, L. J., *Journal of A. M. A.*, 1921, lxxvii, 39.

2 Howland, John and Kramer, Benjamin, *Transactions of the American Pediatric Society*, 1922, xxxiv, 204.

exceed the value 30. When healing takes place the concentration of each element approaches the normal so that the value of the product is 40 or above. Since the calcium concentration in normal serum rarely exceeds 11 mg. per 100 c.c. of serum and that of inorganic phosphorus does not go beyond the upper limit of 6 mg. in the same volume of serum the product practically never exceeds the value 66.

The present study was undertaken:

1. To test the curative action of direct sunlight upon rachitic changes in the bones of children.

2. To determine whether the pigmented skin of the negro interfered with the therapeutic action of the sun's rays.

3. To demonstrate whether direct sunlight is capable of producing as definite an increase in the concentration of calcium and inorganic phosphorus in the serum as occurs with the agents above named.

Although Hess and Unger[1] claim to have produced healing of the rachitic process in the bones in from four to six weeks, Hess and Gutman[3] failed to find any significant changes of the inorganic phosphorus of the whole blood when children were exposed to sunlight for the same period.

Seven colored children suffering from active rickets were studied. Radiograms of all the extremities were taken before treatment was begun and at intervals during the treatment. The serum was also analyzed for calcium and inorganic phosphorus. The children were fed on milk dilutions and orange juice, a diet which, from previous experience, we have found will not itself cure rickets. The children were exposed to direct sunlight beginning with periods of fifteen minutes duration, then one half hour and finally one hour daily. In spite of intense pigmentation of the skin of some of the children, definite evidence of healing could be demonstrated in each instance by means of the radiogram during the third week of treatment. This was well marked by the end of the third week.

Table I shows that in every instance before treatment was begun the product of the calcium by the phosphorus concentration of the serum did not exceed 30, indicating the existence of active rickets. A marked change in the concentration of inorganic phosphorus occurred regularly within fourteen days

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[3] Hess, A. F., and Gutman, M. B., *Journ. of A. M. A.*, 1922, lxxviii, 29.

and the normal level was reached within three weeks so that, at this time, the product approximated or even exceeded the value 60. Whenever the calcium concentration of the serum was low at the beginning of treatment it rapidly rose to the normal level during treatment.

CONCLUSIONS

I. The systematic exposure of children to the direct rays of sun regularly brings about healing of the rachitic process in the bones.

II. The pigmented skin of the negro does not interfere with the therapeutic action of the sun's rays.

III. The changes in the concentration of calcium and inorganic phosphorus are identical with those which follow the administration of cod liver oil or radiation with the mercury vapor quartz lamp.

IV. The action of sunlight is as prompt, if not more so, than that of other curative agents.

CALCIUM AND INORGANIC PHOSPHORUS CONCENTRATION OF SERUM OF RACHITIC CHILDREN BEFORE AND AFTER EXPOSURE TO SUNLIGHT

Name	Color	Age	Date	Calcium mg. per 100 of serum	Phosphorus mg. per 100 of serum	Product	
M. N.	Brown	6 mo.	6-10-22	8.8	2.8	30	Marked rickets.
			8-2-22	11.1	5.0	55	Definite healing.
M. D.	Black	1 yr.	8-8-22	11.0	3.0	33	Moderate rickets.
			8-18-22	10.5	4.2	44	
			8-24-22	11.2	5.9	66	Healed rickets.
G. F.	Dark brown	9 mo.	8-11-22	10.6	3.0	32	Rickets.
			8-24-22	10.3	5.0	51	
			9-1-22	10.1	4.7	47	
			9-7-22	9.2	5.9	54	Healed rickets.
R. F.	Black	10 mo.	8-11-22	9.3	2.8	26	Active rickets.
			8-24-22	10.3	4.1	42	
			9-1-22	10.1	5.7	58	
			9-7-22	9.8	6.4	64	Definite healing.
A. J.	Dark brown	15 mo.	8-16-22	9.3	2.5	23	Rickets.
			8-24-22	9.4	3.2	30	
			9-1-22	9.3	4.1	38	
			9-7-22	9.8	5.9	58	Healing.
E. C.	Black	16 mo.	8-18-22	9.8	2.5	25	Marked rickets.
			9-1-22	10.1	3.8	38	
			9-7-22	9.2	6.5	60	Definite healing.
L. Y.	Black	10 mo.	8-21-22	7.9	3.1	25	Rickets.
			9-7-22	9.0	5.1	46	Healing.

Child treated in the ward for three weeks. Radiogram of the extremities at the end of this time same as on June 10. Child then exposed to sunlight for three weeks, diet unchanged. Definite healing after 14 days.

44 (2004)

Pharmacodynamic reactions of erectile tissue and the dorsalis penis artery.

By DAVID I. MACHT.

[*From the Pharmacological Laboratory and Brady Urological Clinic, Johns Hopkins University, Baltimore, Maryland.*]

The physiology and even the anatomy of erectile tissue has never been satisfactorily investigated and our knowledge on the subject is still very meager. In connection with a pharmacological investigation of *aphrodisiac* drugs the author thought it desirable to inquire into the pharmacological behavior of erectile tissue as well as of the dorsalis penis artery. After long experimentation a method of studying these tissues has been developed and the effects of various drugs on the same were investigated. In the present research isolated surviving pieces of copora cavernosa and spongiosa of the dog were kept alive in warm oxygenated Locke solution under special conditions and the response of the preparations to various drugs was studied. In the case of the dorsalis penis artery, rings of the dog's artery were employed.

The modern anatomist in tracing the finer structure of the nervous system resorts to pharmacodynamic reactions for the determination of the origin of different parts of the sympathetic nervous system. Thus, for instance, a pharmacological response to adrenalin is an indication that the particular muscle preparation studied is innervated by the true or thoracico-lumbar sympathetic system; while a response to such drugs as pilocarpin and atropin indicates an innervation coming from the parasympathetic or bulbo-sacral sympathetic system.

In the present investigation it was found that both erectile tissue preparations and preparations of the dorsalis penis artery responded with contraction or relaxation as the case might be on treatment with epinephrin and ergotoxin. On the other hand numerous experiments made with pilocarpin, physostigmin, atropin and other so-called "parasympathetic" poisons failed to elicit any response in either the erectile tissue or dorsalis penis artery preparations. In view of these results, repeatedly and consistently obtained, it appears that both corpora cavernosa

and spongiosa as well as the dorsalis penis artery of the dog are supplied by nerve filaments belonging to the true sympathetic system and not to the parasympathetic system. Full details to appear in the *Journal of Urology.*

45 (2005)

The influence of the cation in the precipitation of the proteins of blood by sodium phosphate.

By PAUL E. HOWE.

[*From the Department of Animal Pathology of the Rockefeller Institute for Medical Research, Princeton, N. J.*]

The precipitation of the globulins of blood with sodium sulfate at 37°C.[1] indicates the presence of critical zones in the curve of precipitation with increasing concentrations of sodium sulfate. Comparison of the results obtained with sodium sulfate at these zones with other procedures for the precipitation of the proteins of blood, by other salts or acidification, showed similar quantitative results. The critical zones occurred at 10.6[2], 14.2, 17.7 and 21.3 per cent. of anhydrous sodium sulfate, *i. e.,* the designated quantity of salt dissolved in 100 c.c. of water at 37°C. These values are approximately 0.75, 1.00, 1.25 and 1.50 molar solutions of sodium sulfate. Furthermore, under similar conditions any given concentration of salt will precipitate the same amount of protein. These observations have been extended to other salts and it has so far been found that a similar relationship holds for each salt; after precipitation begins there is a constant difference in the concentration of salt for the succeeding fractions. Precipitation of fibrinogen ends at approximately the concentration of salt found by Lewith[3] for the beginning of the precipitation of euglobulin,—observations which were correlated on the basis of equivalent concentrations by Hofmeister[4]. The difference in concentration between the various fractions is not necessarily the same for all salts, *e.g.,* for magnesium sulfate it is 0.375 mol.

[1] Howe, Paul E., *Jour. Biol. Chem.,* 1921, xliv, 93.
[2] Howe, Paul E., *Jour. Biol. Chem.,* 1922, liii, 479.
[3] Lewith, S., *Archiv. f. Exper. Path. u. Pharm.,* 1887, xxiv, 1.
[4] Hofmeister, F., *Archiv. f. Exper. Path. u. Pharm..* 1887, xxiv, 257.

Sodium phosphate at P_H 7.0 completes the precipitation of fibrinogen at an approximately molar PO_4 solution and the difference for subsequent fractions is 0.25 mol. By varying the proportions of mono-sodium and di-sodium phosphate to obtain solutions of similar molecular concentration with regard to the PO_4 radicle, but with varying hydrion concentrations, it was found that higher molecular concentrations were required for the precipitation of the various protein fractions. By adjusting the concentrations of the phosphate solution at each P_H to concentrations of sodium which were equal to the sodium content of solutions which were 1.0, 1.25, 1.50 and 1.75 molar with regard to the phosphate radicle at P_H 7.0, it was found that between approximately P_H 8.0 and 5.8 essentially the same amount of protein was precipitated as at P_H 7.0. If the precipitations be made with concentrations of sodium phosphate at P_H 7.0 which are such that they are equal in sodium concentration to the sodium sulfate solutions, essentially similar results are obtained with the two salts for each protein fraction. In fact, the sodium concentrations of the fractional molar concentrations with relation to the phosphate radicle given above differ only slightly from those of concentrations found for sodium sulfate.

When the proteins of serum were precipitated with the different sodium phosphate solutions no precipitation occurred until the equivalent of 1.25 molar phosphate at P_H 7.0 was added, *i.e.*, no fibrinogen was present. If the serum of the new-born calf was used, precipitation did not occur until the equivalent of 1.75 molar phosphate at P_H 7.0 had been added, or only a slight precipitation occurred at 1.50 molar. On the other hand, if the *plasma* of the new-born calf was used, precipitations occurred at the equivalent of 1.0 molar phosphate and no increase was observed until a concentration above 1.50 molar was reached, *i.e.*, fibrinogen, euglobulin and pseudoglobulin I were absent from serum and fibrinogen was present in the plasma.

In all of these cases we were dealing with a constant concentration of sodium but with varying concentrations of the phosphate radicle—a range of approximately 0.8 mol. for any range of the hydrion concentrations. It appears, therefore, that the precipitation of the proteins of blood at hydrion concentrations below the neighborhood of the isoelectric point is primarily due to the cation.

46 (2006)

On the absence of isoagglutinins in mice.

By E. C. MacDOWELL and J. E. HUBBARD.

[*From the Station for Experimental Evolution, Cold Spring Harbor, Long Island, New York.*]

The number of units of inheritance, or genes that are known in mice, is already so much in excess of the number of those known in any other mammal that there is offered a strong inducement to make intensive studies in order to increase the number of genes to the point where the deeper study of the genetic mechanism of a mammal may be undertaken. A possible field for the search for new characters was suggested by the claims of Von Dungern and Hirschfeld[1], Learmonth[2], and Ottenberg[3] that blood groups in man depend in their inheritance upon simple Mendelian factors. Besides being of interest from the standpoint of mouse genetics and of the technique of experimental transplantations, the discovery of blood groups in mice would lead to a genetic investigation that could provide an experimental basis for the study of the inheritance of blood groups in man.

The tests made upon rabbits and steer by Ottenberg and Friedman[4] are said to reveal the presence of blood groups in these animals. On the other hand, other investigators (Hektoen[5], Ingebristen[6], Fischbein[7], and Rohdenburg[8]), using a variety of animals (cats, dogs, sheep, swine, cattle, horses, rabbits, guineapigs, rats, and frogs), have failed to find any evidence of blood groups. In certain cases agglutinations were found, but these did not appear to be grouped.

Such negative results did not promise well for the discovery of blood groups in mice, but the availability of a greater number

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[1] Von Dungern and Hirschfeld, *Zeit. f. Immunitatsforschung*, 1910, vi, 284.

[2] Learmonth, J. R., *Jour. Genetics*, 1920, x, 141.

[3] Ottenberg, R., *Journ. Immunology*, 1921a, vi, 363; *Journ. Am. Med. Asso.*, 1921, lxxvii, 682, and 1922, lxxviii, 873.

[4] Ottenberg and Friedman, *Journ. Exp. Med.*, 1911, xiii, 531.

[5] Hektoen, *Journ. Infect. Dis.*, 1907, 297.

[6] Ingebristen, *Münchener med. Woch.*, 1912, lix, 1475.

[7] Fischbein, *Journ. Infect. Dis.*, 1913, xii, 133.

[8] Rohdenburg, G. L., Proc. Soc. Exp. Biol. and Med., 1920, xvii, 82.

of different races than had been used in testing the other animals favored the chances of a positive result.

The following races of mice were used in the present experiments: (1) Japanese Waltzers (Lambert strain), which originated from a single pair of mice isolated in 1906 and intensively inbred ever since; (2) ten lines inbred three or four generations from Lathrop stock by Dr. C. C. Little in his experiments with X-rays; (3) Lathrop stock mice untreated with X-rays; (4) Albinos of a vigorous strain inbred since 1912 by Dr. H. J. Bagg of the Memorial Hospital; (5) from Dr. L. C. Dunn of the Agricultural Experiment Station at Storrs, Conn., three selected piebald families, inbred brother by sister for three years, descendants of an old race originally at the Bussey Institution of Harvard University, later in the hands of Dr. J. A. Detlefson, and later in the possession of a fancier; (6) Dilute Browns, a race inbred by Dr. Little since 1909; (7) Storrs-Little, a race of pink-eyed blacks, derived from the preceding race with the introduction of a single unrelated animal and subsequently crossed back to the pure dilute brown for five generations; (8) Black-eyed Whites, inbred brother by sister for a year by Dr. L. C. Strong of St. Stephens College, mice coming from a race kept pure since its introduction from England in 1913 at the Bussey Institution; (9) Cold Spring Harbor wilds, comprising two sets of wild mice taken at two separated places in Cold Spring Harbor, both places being at some distance from the Laboratory; (10) Storrs wilds, raised from wild mice collected at Storrs, Conn.; (11) Waltzers; a stock from a back cross between Japanese Waltzers and Bagg Albinos made by Miss E. M. Vicari. The use of these various races was made possible by the cooperative spirit of the following investigators: Drs. Little, Dunn, and Strong, Mr. Gates and Miss Vicari.

METHODS

In order to secure enough serum it was necessary to kill the mouse. The most successful method proved to be to etherize, suspend it by its tail and cut its throat suddenly with scissors, catching the blood in a centrifuge tube by means of a paraffined funnel. After the blood clotted, the serum was separated in an electric centrifuge and pipetted off into a small test tube. To obtain samples of cells, the tip of a mouse's tail was cut off and

small amounts of blood taken with a pipette and added to about a c.c of a 0.7 per cent. salt solution. Based on the light pink color, cell suspensions of a suitable concentration were obtained. This method was used because the main problem necessitated a procedure that did not require killing the mouse to be tested. Each test was made by microscopic examination of a hanging drop of equal quantities of cell suspension and serum; the observations were made within an hour.

From the various races of mice forty-eight sera were made, and a total of 300 samples were tested; the total number of different combinations of cells and sera was 1,180. Of these combinations only two showed any agglutination. These two cases were combinations of cells from two wild mice and serum from a Storrs-Little pink-eyed black. Repeated tests with cells from the same mice and serum from mice related to the one that gave the first serum failed to give further agglutinations.

Since one mouse yields such a small amount of serum, at best 2 c.c., it was thought that some other serum, obtainable in larger quantities might have advantages in testing for differences in the blood of different strains of mice.

To this end four guinea-pig sera were used in making 180 tests; four sheep sera were used in making 136 tests, and nine white rat sera were used in making 276 tests. In every case the guinea-pig and sheep sera showed unquestionable agglutination, and in no case did the rat sera show any signs of agglutination, although the whole range of mouse races was tested with each of the sera.

These results appear to be conclusively negative from the standpoint of finding blood groups in mice that could be utilized in a genetic study; from the standpoint of the agglutinations between different species, it appears that guinea-pig and sheep differ from rats in the reactions of their sera to mouse cells.

47 (2007)

Dinitrosalicylic acid as a reagent for blood sugar.

By JAMES B. SUMNER and V. ARVIN GRAHAM.

[*From the Department of Physiology and Biochemistry, Medical School, Cornell University, Ithaca, New York.*]

The reagent[1], in use at Ithaca since 1921 for the estimation of sugar in normal and diabetic urine, has been applied to blood.

The blood proteins are perfectly precipitated by the addition to 1 c.c. of blood, laked with 2 c.c. of water, of 4 c.c. of the neutral sodium salt of dinitrosalicylic acid (2.94 per cent.), followed by 2 c.c. of dilute sulphuric acid (0.40 N). Filtration is rapid. Excess of oxalate does not interfere. Three c.c. of the filtrate are heated in a Folin sugar tube in boiling water for three minutes to remove the dissolved oxygen. One c.c. of 3 per cent. sodium hydroxide is then added. This last reagent is made up saturated with sodium chloride to prevent oxygen from dissolving in it.

The test tube is heated for 10 minutes more, cooled and diluted to 25, 50, or 100 c.c. volume. The standard is prepared by heating 2 c.c. of a 0.015 per cent. glucose solution with 1 c.c. of neutral 1.78 per cent. sodium dinitrosalicylate for 3 minutes, adding 1 c.c. of the alkali and heating for 10 minutes.

The method is convenient; one standard will keep all day and can be used with bloods containing from 50 to 300 mg. of sugar per 100 c.c.

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[1] Sumner, J. B., *Jour. Biol. Chem.*, 1921, xlvii, 5.

48 (2003)

The flora of the human alimentary tract: stomach, duodenum,
jejunum.

By B. ARONOVITCH, WARREN COLEMAN and MAX EINHORN.

[*From the Department of Medicine, University and Bellevue
Medical College and the Third Medical Division of
Bellevue Hospital, New York City.*]

The flora of the human alimentary tract has been the subject
of numerous investigations, but such studies during life have, of
necessity, been confined to the floras of the stomach and duode-
num and of the feces, except when operations or post-operative
fistulæ have permitted approach to other portions of the tract.
Obviously, many levels of the alimentary tract have thus been left
unexplored.

To obtain a complete picture of the flora of the human alimen-
tary tract in the living subject, and of the changes which the
flora undergoes as the intestinal contents travels downward, the
method pursued must permit the extraction at will of specimens
from any level of the intestine. The introduction of the Einhorn
intestinal tube makes it possible to fulfill these conditions. (It
may be stated parenthetically that we do not yet know the ex-
treme depth from which intestinal contents may be extracted.
We have been able to obtain material from a distance of 135
inches from the lips and hope to make extractions from as far as
the ileo-colic valve).

Our studies were begun in the fall of 1920. So far, we have
confined our attention to the floras of the stomach, duodenum
and jejunum. Certain technical difficulties which we encoun-
tered may be referred to:

Coiling of the tube in the stomach or below has occurred so
often we have concluded that the true position of the tube can
be ascertained only through roentgenograhpic examination. In
all of our subjects, such examinations have been made after
each extraction from below the stomach.

Unless the distal end of the intestinal tube is kept closed until
the moment of extraction, it is impossible to tell when, or at what
level, intestinal contents first enters the tube. Once it enters, the
conditions obtaining within the intestine itself are removed and
the development of the flora is unrestrained.

Extraction. All extractions were made in the morning before the subject had taken water or food, approximately 15 hours after the last meal.

Extractions from the stomach were made one hour after the tube was swallowed; extractions from the duodenum (below 24 inches) were made three hours after swallowing; for longer tube-lengths, intervals of eighteen hours to thirty-six hours were allowed.

All specimens were delivered to the laboratory within half an hour and at once submitted to the various procedures.

Eight subjects were utilized for the study; some of them were under observation for several months. All were patients in Bellevue Hospital. Sub. I suffered from chronic arthritis and Sub. II from arthritis deformans. (The attempt was made to utilize patients from the Lenox Hill Hospital also, but the distance of this hospital from the laboratory caused too much delay between the extraction and the study of the specimens).

Results. The results obtained in the eight subjects are summarized below. The Table presents the details of the examinations of two subjects at three levels of the alimentary tract.

Stomach Contents—Number of Organisms. The number of viable organisms and spores in the stomach contents were uniformally low except in Subject II in whom the numbers, exclusive of spores, were always high. (It may be added that there was no obvious discharge of pus from this subject's mouth).

Varieties of Organisms. Eleven different varities of organisms were identified in the gastric contents. Some seven varieties of Gram-positive, spore-bearing bacilli, some of them pleomorphic, were isolated but not identified. The greatest variety of bacteria was found in Sub. I with relatively small total numbers.

The occasional presence in the stomach of organisms, commonly identified with the intestine is probably due to regurgitation from the duodenum.

Cultural Properties. Acid production was the rule in dextrose-broth and litmus-milk. The production of gas was not constant and the quantity was small. Milk was coagulated and the curd was partially or completely digested. Loeffler's serum showed only slight digestion occasionally. Gelatin was partially or completely liquified.

Duodenal Contents. (28 inches from lips). *Number of organisms.* Except in Sub. II, the numbers of viable organisms and spores in the duodenal contents also are small, yet a slight tendency to an increase in numbers is noticeable. In Sub. II the increase is definite. The wide fluctuations in the numbers on different occasions in Sub. II was not discovered.

Varieties Isolated. There is no increase in the varieties of organisms isolated from the duodenal contents, but the diversification of the flora in the individual subject is, as a rule, greater.

The Gram-positive, spore-bearing bacilli are present with the same constancy. Staphylococcus albus has disappeared in two subjects. Streptococci have appeared in two additional subjects and bacteria, commonly classified as intestinal, such as *B. Coli, paracolon bacillus, B. acidophilus* and the *enterococcus,* are found with greater frequency.

Cultural Properties. Acid production is still the rule in dextrose-broth and litmus milk. Gas production, though limited in quantity, is slightly more evident. Milk is coagulated and the curd is digested. Loeffler's medium exhibits slight digestion. Gelatin is more frequently liquefied.

Jejunal Contents. (34 inches to 135 inches). *Number of organisms.* In Sub. I, the number of viable organisms, at depths of 87 inches to 135 inches from the lips is strikingly small. In Sub. II, at depths of 90 inches to 115 inches, a decided increase in numbers has occurred, though wide fluctuations may still be noted. In Sub. IV there is a progressive increase to the depth of 60 inches. In Sub. VII there was no increase in numbers down to 60 inches, and in Sub. III the increase was negligible. In only two subjects, Sub. I and Sub. II, were extractions made from below 60 inches.

Cultural Properties. The production of acid in dextrose-broth and litmus-milk is still the rule. Gas is produced more frequently and in greater quantity, especially by flora from the deeper levels. Litmus-milk is coagulated and the curd is digested. Loeffler's medium is only rarely and partially attacked. Gelatin is partly or completely liquified.

Varieties Isolated. Fifteen varieties of organisms were isolated from the jejunal contents, *B. mucosus* capsulatus and *B. proteus* having appeared for the first time. But it may be noted that the total number of examinations of jejunal contents was

	GASTRIC CONTENTS		DUODENAL CONTENTS		JEJUNAL CONTENTS	
	Subject No. 1	Subject No. 2	Subject No. 1	Subject No. 2	Subject No. 1	Subject No. 2
Length of tube	Tube in stomach		28 inches		120 inches	115 inches
Number of bacteria per c.c. {aerobic / anaerobic}; Number of spores	200 >10 / 500 >10 / Few Few	310,000 1,430,000 / 144,000 2,400,000 / 30 Few	>100 >10 / >100 >10 / Few Few	2,420,000 10,000 / 2,350,000 45,000 / Few Few	100 / 190 / Few	13,000,000 / 3,260,000 / Few
Organisms isolated	B. coli Paracolon B. aureus Staph. aureus Staph. albus B. subtilis Gram + bacilli (spores) Yeast	B. coli Streptococcus Gram—coccus Staph. aureus Gram + bacilli (spores)	B. coli Paracolon Gram—coccus M. tetragenus Gram + bacilli (spores) Yeast	Streptococcus Gram—coccus Staph. aureus B. acidophilus Gram + bacilli (spores) Yeast Enterococcus Diphtheroid bacillus	B. coli Paracolon Gram + bacilli (spores) Yeast	B. coli Streptococcus B. alkalig. fæcalis Staph. aureus Staph. albus B. acidophilus Gram + bacilli (spores) Yeast Enterococcus B. lact. ærog. B. cloacæ B. subtilis
Dextrose-broth fermentation tube	Slightly acid	Acid	Slight or no acidity	Acid	Acid Gas	Acid Gas
Litmus-milk	Acid Gas Coagulation Slight digestion	Acid Gas Coagulation	Acid Gas Coagulation Digestion	Acid Gas (a little) Coagulation Digestion	Acid Gas Coagulation	Acid Gas Coagulation Digestion
Loeffler's medium	Slight or no digestion	No digestion	No digestion	No digestion	No digestion	No digestion
Gelatin	Slight or no liquefaction	Complete liquefaction	Slight liquefaction	Complete liquefaction	Slight or no liquefaction	Complete liquefaction

greater. The diversification of the flora became greater in some of the subjects as lower levels of the jejunum were reached; in the majority of them bacilli of the colon group assumed greater prominence. The Streptocci persist in Sub. II and the question arises whether their continued presence indicates a definite local-ization (infection) in the alimentary tract.

ABSTRACTS OF COMMUNICATIONS

Special June meeting.

Minneapolis, Minnesota, June 12, 1922.

49 (2009)

The determination of iodine in large samples of foodstuffs.

By J. F. McCLENDON and O. S. RASK (by invitation).

[*From the Laboratory of Physiological Chemistry, University of Minnesota Medical School, Minneapolis, Minnesota.*]

The method of Kendall was found satisfactory for estimating small amounts of iodine as low as 0.02 mg. where destruction of large amounts of organic matter was not involved. If, however, the size of the sample is increased there is a corresponding de-crease in accuracy. In order to reduce the bulk of material the following processes were carried out: Cereal grains were made into beer without removal of any solid matter and the alcohol volatilized after making the beer alkaline. In the combustion, the fumes were passed through an alkaline solution to catch the iodine. After the iodine was in the form of I_2 it was shaken out with carbon tetrachloride and then this carbon tetrachloride shaken with a dilute solution of SO_2 to get the iodine into water solution again as iodine. By these processes both organic and inorganic inert constituents may be eliminated.

50 (2010)

The height-weight index of the newborn infant.

By RICHARD E. SCAMMON.

[*From the Department of Anatomy, University of Minnesota, Minneapolis, Minnesota.*]

The material used in this study consisted of the records of the heights (or lengths) and weights of some 4,200 living newborn children from a number of European clinics. The lengths were all in centimeters, the weights in grams. The cases were grouped according to their lengths in centimeter intervals, the group of shortest infants including those from 47 to 48 cm. and the group of longest cases including those from 56 to 57 cm. in length. The males and females of each group were considered separately. The average weight and the standard deviation, the probable error and the coefficient of variability of the weight were determined for each group. The ponderal or height-weight index of the average weight for each group was also worked out according to the formula:

$$\text{P. I.} \ \frac{\text{Weight}}{\text{Height}^3} \times 1000.$$

These indices were determined both on the pound-inch (avoirdupois) and the centimeter-kilogram (metric) basis. The results obtained are shown in part in the graph below and may be summarized as follows:

(1) With the increase in total body length in the latter part of prenatal life the height-weight index drops slowly, there being a decline of 1.5-2.5 per cent. (avoirdupois index) or 0.4-0.8 per cent. (metric index) between the average for 47 cm. and that for 50 cm. of total body length. In infants over 50 cm. in total body length the index declines much more rapidly, falling from an index of the average of 89.50 per cent. for males and 88.12 per cent. for females (avoirdupois) at 50 to 51 cm. to one of 79.91 per cent. for males and 80.08 per cent. for females at 56 to 57 cm. of body length. The drop in the metric index for the same period is from 25.99 per cent. to 22.94 per cent. for the males and from 25.60 per cent. to 23.24 per cent. for the females. This agrees with Bardeen's findings[1] that longer newborn infants

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[1] Bardeen, C. R., Publ. 272, Carnegie Inst. of Washington, 1919.

are relatively lighter than shorter ones, although the values are slightly below those reported by Bardeen. It seems significant that this drop is accentuated after the infant reaches the length of 50 cm. which is generally regarded as a criterion of the completion of the normal span of intrauterine life. Since the ponderal index drops very rapidly with increasing length after 50 cm. the usual empirical formulæ for body length and body height in the fetal period will not hold for these longer infants.
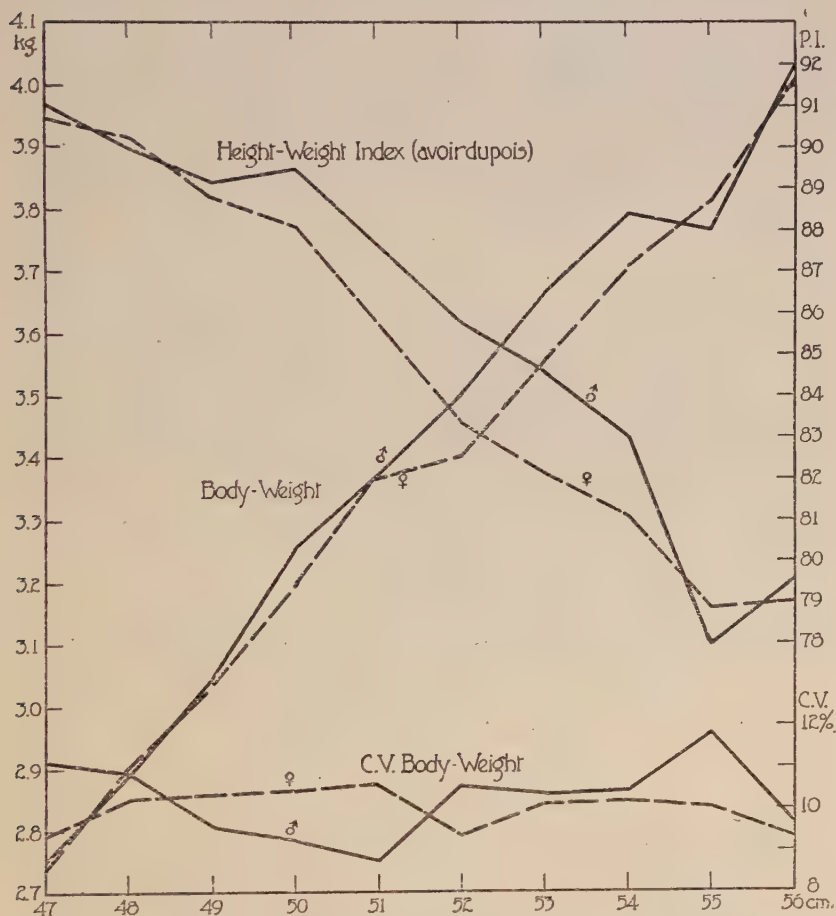
A graph illustrating the relation between body length (or height) and body weight in a series of 4,208 living, new-born children. The curves show the average body weight, the coefficient of variability of body weight and the height-weight index for centimeter intervals of body length from 47 to 57 cm. The males are indicated by solid, the females by broken lines.

(2) In the majority of the groups of cases the height-weight indices of the males were above those of the females. However, the differences were so small that their significance is questionable.

(3) When the cases are arranged in groups according to centimeter intervals of body length the coefficients of variability in weight of these groups are found to range from 9.5 to 12.0. There is no regular change in the coefficients with increasing body length and no constant sex difference in the coefficient is noted.

The above conclusions were drawn from a study of the ponderal index as determined from the average weight for a given length. The difference between this figure and the average of the *individual indices* for a given length was tested by determining these two values separately for the 50 to 51 cm. group which included 996 cases. In this group the height-weight index of the average height and weight was 25.99 per cent. (metric) for the males and 25.60 per cent. (metric) for the females. The average height-weight index was 26.01 per cent (metric) for the males and 25.60 per cent. (metric) for the females. The average ponderal index of the males showed a standard deviation of 2.41 and a coefficient of variability of 9.27 while that of the females showed a standard deviation of 2.26 and a coefficient of variability of 8.85.

51 (2011)

Experimental demonstration of the entire course of four descending tracts[1] by a single alcoholic injection in the mid-brain of the cat.

By A. T. RASMUSSEN.

[*From the Department of Anatomy, Medical School, University of Minnesota, Minneapolis, Minnesota.*]

By trephining a hole 2 cm. in diameter, 1 cm. anterior to the occipital crest and 2 cm. from the median line, and retracting the occipital pole of the cerebral hemisphere of the cat, the superior colliculus was exposed and about two drops of 95 per

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[1] Fasciculus longitudinalis medialis, fasciculus tectospinalis, fasciculus rubrospinalis, radix mesencephalica trigemini.

cent. alcohol injected into the nucleus ruber with a fine hypodermic syringe. The needle should enter the surface at about the middle of the colliculus and be directed slightly medially and at right angles to the long axis of the brain stem to a depth of 1 cm. After the usual two weeks, the animal was killed and the brain carried through the regular Marchi method.

As the accompanying illustrations show, it is possible in this manner to cause degeneration of the entire extent of four descending tracts, two of which are not easily recognizable on normal material and yet are functionally of great importance. These fiber bundles in the cat are situated in the same relative position as in man.

Although the exact course (especially peripherally) of the mesencephalic root of the trigeminal nerve has been much debated[2], it is clearly evident that its exit through the pons is closely associated with the motor root of the trigeminal nerve.

The fibers from the nucleus of the medial longitudinal fasciculus (degenerated only on one side) and those from the tectum mesencephali (degenerated mostly on the contralateral side) occupy distinct regions throughout their entire course in the cat. Numerous other experiments in which we have limited the lesion to the tectum of the mid-brain have failed to show that any fibers from this region enter what is strictly the medial longitudinal fasciculus. The lower termination of the tectospinal fasciculus has been variously given[3]. In this and the other experiments referred to above, fibers of this fasciculus cannot be followed farther than the upper part of the 7th cervical segment. It is very probable that the fibers followed below this level by others belong to some other tract such as the medial longitudinal fasciculus, which extends throughout most of the spinal cord. It is the large number of ascending and descending fibers from the vestibular nuclei that make it appear that the medial longitudinal fasciculus is partly mixed with the tectospinal. A clearer designation of the various fiber tracts now generally considered as part of the medial longitudinal fasciculus is highly desirable.

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[2] Allen, W. F., *Jour. Comp. Neur.*, 1919, xxx, 169.

[3] Collier, J. and Buzzard, F., *Brain*, 1901, xxiv, 177.

The rubrospinal fasciculus, from its origin, size and extent, as well as from clinical studies[4], should be regarded as an important motor pathway by which both the corpus striatum and cerebellum may exert its influence on lower motor centers. While widely separated from the pyramidal tract above the pyramidal decussation, below this point it is more and more closely associated with the lateral (crossed) pyramidal tract. As was to be

Fig. 1
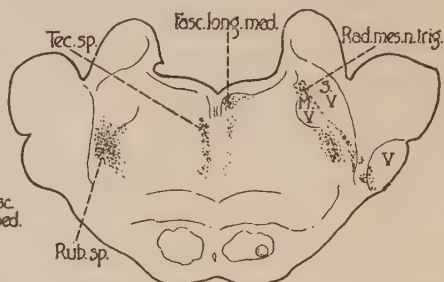
Fig. 4

Fig. 2
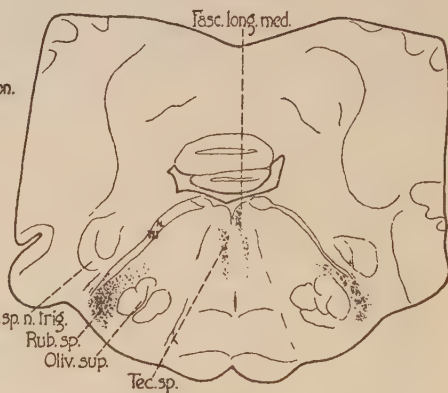
Fig. 5
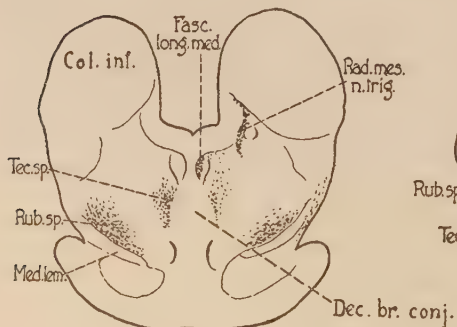
Fig. 3
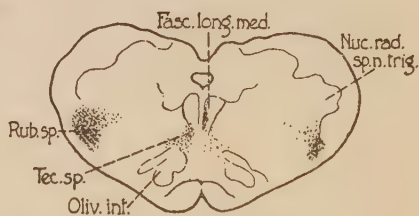
Fig. 6

[4] Hunt, J. R., Jour. Nerv. and Ment. Dis., 1916, xliv, 437; 1917, xlvi, 211; Brain, 1918, xli, 302.

expected, the physiological effects of the injection of one nucleus ruber was much the same as a large lesion in the opposite cerebellar hemisphere.

Nuc. rad. sp. n. trig.—
Rub. sp.—
Tec. sp.—
Fasc. long. med.
Dec. pyr.

Fig. 7

Fasc. long. med.
Cort. sp. lat.
Rub. sp.
Tec. sp.—
Fig. 11, 2nd. Cervical

Cort. sp. lat.
Rub. sp.
Tec. sp.—
Fasc. long. med.

Fig. 8

Cort. sp. lat.
Rub. sp.
Fasc. long. med.
Fig. 12, 7th. Cervical

Cort. sp. lat.
Rub. sp.
Tec. sp.—
Fasc. long. med.

Fig. 9

Rub. sp.
Fasc. long. med.
Fig. 13, 7th. Thoracic

Rub. sp.

Fig. 14, 6th. Lumbar

Fasc. long. med.
Cort. sp. lat.
Rub. sp.
Tec. sp.—
Fig. 10, 1st. Cervical

Rub. sp.

Fig. 15, Sacral

ABSTRACTS OF COMMUNICATIONS

Sixth meeting.

Minneapolis, Minnesota, October 18, 1922.

52 (2012)

The fermentation of glucose by Fusarium lini.

By ARTHUR K. ANDERSON and J. J. WILLAMAN.

[*From the Division of Agricultural Biochemistry, University of Minnesota, Minneapolis, Minnesota.*]

In a study of the biochemistry of *Fusarium lini,* the organism which causes flax wilt, its action on a nutrient solution containing glucose as the only source of carbon, has been investigated. Tochinai[1] in Japan has shown that *Fusarium lini* grows well on several of the common carbohydrates as a source of carbon. Of these he found inulin and glucose to give the best growth. In fermentation tests he found gas produced. Glucose produced gas in largest quantities, while galactose produced only traces. He considers this gas to be entirely CO_2. He suggests it is very probable that the wilt of the flax plant is due to the production of gas in the vascular system of the plant which interferes with transpiration.

In the present work a detailed study of the action of *Fusarium lini* on glucose has been made. The nutrient solution used had the following composition:

$$NH_4NO_3 .... 1.00 \text{ g.}$$
$$KH_2PO_4 .... .50 \text{ g.}$$
$$MgSO_4 .... .25 \text{ g.}$$
$$Glucose .... 20.00 \text{ g.}$$
Distilled water to make 1,000 c.c.

The reaction of this medium was adjusted to the desired P_H by the addition of HCl or NaOH solutions. One hundred c.c. were placed in 300 c.c. Erlenmeyer flasks with 2-hole rubber stoppers fitted with cotton plugged glass tubes and rubber tubing with pinch clamps to prevent the escape of any gasses produced. These

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[1] Tochinai, Y., *Annals of the Phytopathological Society of Japan,* 1920, i, 1; *Byochūgai Zasshi,* 1921, viii, 2 (Japanese).

flasks were sterilized and inoculated with a definite volume of a spore suspension of *Fusarium lini* and incubated at 28 to 30 C.°. In the experiment here reported 15 flasks were used, the P_H being adjusted to 5.545, which is within the optimum reaction for this organism.   These flasks were divided into sets of three each, and at intervals sets were removed and analyzed.   Flask 1 was used for determining alcohol; flask 2 for mycelium, glucose, and lead precipitate; and flask 3 for mycelium and P_H.   The CO_2 was determined on each flask at frequent intervals to avoid the development of pressure.   The mycelium was separated from the medium by filtration onto a gooch.   The carbon in the original culture, in the mycelium, and in the lead precipitate, was determined by wet combustion; alcohol was determined by aeration into concentrated H_2SO_4, oxidation with potassium dichromate to acetic acid, and distillation and titration of the latter; and glucose by the picramic acid method.   Since in a previous experiment succinic acid had been identified as a product of the action of the organism on glucose, it was thought that lead acetate would precipitate any of this, hence the determination of the lead precipitate.

Chart I shows the results of the experiment.   It is evident that over 90 per cent. of the glucose can be accounted for and that
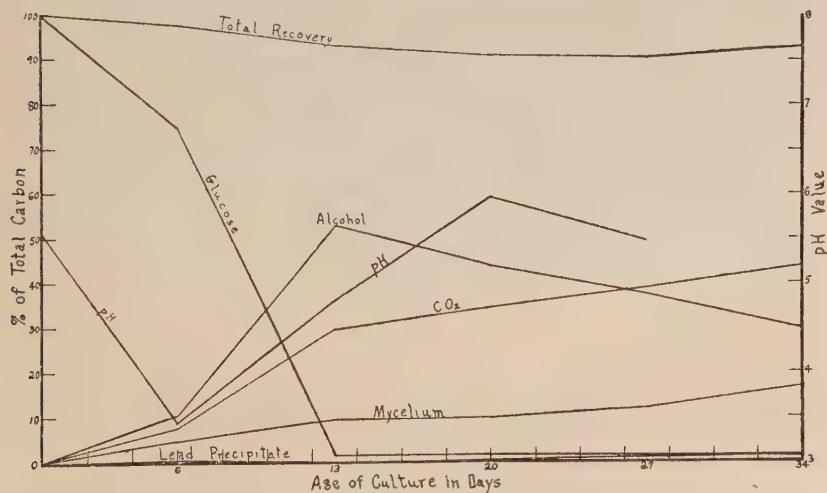
CHART I. Curves showing the percentage of carbon in the products of metabolism of *Fusarium lini* on glucose at different stages of growth.   Change in P_H is also shown.

ethyl alcohol and CO_2 are the main products of the action of the organism on the glucose. This suggests an analogy to yeast fermentation. In the conventional equation for yeast fermentation, the carbon in the alcohol and in the CO_2 is in the ratio of 66.33; in the present data it is in the ratio of 62.35, which is a good agreement considering the fact that alcohol is consumed by *Fusarium lini,* as will be mentioned below. The other by-products of yeast fermentation, such as glycerol, acetone, and succinic acid, have not been identified in the present study, with the exception of one instance where succinic acid was found in small amounts. It is interesting to note that the quantity of alcohol increases until the glucose is gone, and that the fungus continues to grow at the expense of the alcohol, using it for growth and metabolism. The change in P_H of the medium is rather marked, and it appears to be due to a selective absorption of ions rather than to a production of organic acid. The lead precipitate is practically negligible and cannot account for any appreciable amount of succinic acid.

The same experiment was performed starting with media at a P_H of 3.685 and 8.960. The results were not essentially different from those here reported.

To prove more definitely that *Fusarium lini* can utilize ethyl alcohol as an only source of carbon, a series has been run in which increasing amounts of ethyl alcohol have been the only source of carbon. Good growth has been observed on cultures containing 4.04 per cent. of ethyl alcohol by volume. There was no growth on a culture containing 5.38 per cent. of alcohol. The best growth occurred where there was about 2 per cent. of alcohol.

We may conclude from the above study that *Fusarium lini* produces ethyl alcohol and carbon dioxide as the main by-products of metabolism when grown on glucose media, and that in the absence of glucose it can utilize the alcohol for metabolism and growth.

53 (2013)

The intraperitoneal transfusion of citrated blood.

By DAVID M. SIPERSTEIN and J. MARTIN SANSBY.

[*From the Department of Pharmacology, University of Minnesota, Minneapolis, Minnesota.*]

These results are based on a study of over 100 rabbits. *Freshly citrated* blood was injected *immediately* into the peritoneal cavity of normal and anemic rabbits. In several instances, citrated pigeon's cells were introduced into the abdominal cavity of rabbits. All the animals were subjected to careful blood studies. They were killed and autopsied at the end of each series of experiments.

We think that freshly citrated blood injected into the peritoneal cavity of rabbits is absorbed, because:

1. Autopsies at various intervals following the operation show that the quantity present in the abdominal cavity rapidly decreases in amount, and that absorption of comparatively large amounts of blood (one-fifth of the total blood volume) is complete in 3-4 hours.

2. Estimations of blood values show definite increase from the time the blood is transfused until the animal is killed.

We think that erythrocytes enter the blood without undergoing any morphological changes, because:

1. Smears, taken at the autopsy, of the fluid in the abdominal cavity show no change in the size, shape or structure of the corpuscles and no evidence of hemolysis.

2. Our experiments show a rise in hemoglobin and cellular elements following transfusions in normal and anemic animals which cannot be accounted for by a mere concentration of the blood.

3. Nucleated corpuscles of pigeons when injected intraperitoneally into rabbits can be recovered from the general circulation in 15 minutes.

We submit the following additional evidence that the erythrocytes function, because:

1. They enter the blood stream very rapidly and are therefore presumably in a functioning condition.

2. The increase in the blood values of our animals persisted for many days.

3. After severe hemorrhage, the animals improved visibly following the transfusion.

4. No hemoglobinuria could be demonstrated at any time.

5. Autotransfusion and retransfusion have been used successfully in man.

6. Clinical experiences with cases of internal hemorrhage as compared with external hemorrhage tends to show that the blood is re-absorbed in a functioning condition.

As a result of our investigations we can draw the following conclusions:

1. The intraperitoneal transfusion of freshly citrated blood in rabbits is a safe procedure, simple to apply and efficient.

2. Absorption of blood from the peritoneal cavity of rabbits takes place very rapidly.

3. The intraperitoneal transfusion in anemic and normal animals apparently causes a sharp rise of blood values during the absorptive period (3-4 hours) which is only temporary. This is followed by a more permanent increase in the blood picture.

4. Studies at the autopsy table together with the blood counts would apparently indicate that the initial rise is due to an actual absorption of red blood cells from the peritoneal cavity and not merely to a concentration of the blood volume.

5. Pigeons' cells injected subcutaneously in rabbits cannot be found in the general circulation. The same blood injected intraperitoneally is absorbed very rapidly.

6. Rabbits' blood probably contains neither hemolysins or agglutinin.

7. The intraperitoneal transfusion of freshly citrated blood acts like a true transfusion and not like the absorption of nutrient material.

It is proposed as a therapeutic method of possible merit.

54 (2014)

Electrical control of polarity in an egg.

By E. J. LUND.

[*From the Puget Sound Marine Biological Laboratory and the Department of Animal Biology, University of Minnesota, Minneapolis, Minnesota.*]

The egg of *Fucus inflatus* is normally shed into the water after low tide. The previous period of exposure to drying during low tide can be imitated by wrapping plants in paper and leaving for about twelve hours, then removing the tips of the plants, which bear the reproductive organs, and floating them in dishes of sea water[1].

The shedding of the eggs can then be allowed to take place upon thin cover glasses to which the eggs adhere securely in about six hours. One of the two cells of the first cleavage gives rise to the frond while the other gives rise to the holdfast. Therefore such a preparation fulfills ideally the necessary conditions for determining the orienting effect of a direct electric current of proper density, upon the longitudinal axis of symmetry in the future plant body.

The cover glasses holding the eggs were placed in the bottom of a special glass trough, through which flowed fresh sea water and an electric current of appropriate density. The threshold value of the fall of electrical potential through an egg, necessary for orientation of the first cleavage is definite and amounts to about .035 volt. Perfect orientation and normal growth only occurs within a relatively narrow range of electrical potential which lies around .025 volts. Higher potentials inhibit cleavage, stop growth or kill the egg. It seems permissible to conclude that the establishment of an electrochemical polarity in the egg is probably an associated condition for the development of morphological polarity, because the physiological mechanism which determines morphological polarity can be controlled and directed by an electric current of external origin. Direct evidence of the existence of such inherent potential differences along the axis in certain eggs[2] and tissues[3] already exists.

[1] Hurd, A. M., *Bot. Gaz.*, 1920, lxx, 25.
[2] Hyde, I. H., *Am. Jour. Phys.*, 1904, xii, 241.
[3] Lund, E. J., *Jour. Exp. Zool.*, 1921, xxxiv, 471.

55 (2015)

The occurrence of multilocular fat cells in the perirenal fat of man.

By A. T. RASMUSSEN.

[*From the Department of Anatomy, University of Minnesota Medical School, Minneapolis, Minnesota.*]

While a number of writers[1] have called attention to the presence in man of a multilocular adipose tissue similar to the so-called hibernating gland of animals, convincing evidence of the structural similarity between these tissues does not seem to have been brought forth.

Gross and microscopic preparations of multilocular adipose tissue in the perirenal fat of a new born and of a child 1½ years old are demonstrated with similar specimens from both the white rat and the American marmot, showing great similarity between this type of adipose tissue from all three sources. Its glandular appearance is striking until examined microscopically. The evidence indicates that these multilocular fat cells are not developmental stages of ordinary fat.

The history, distribution and functional significance of this brown gland-like fatty tissue leads to the conclusion that there are not sufficient data to warrant taking seriously the suggestion that it may be an endocrine organ of importance in deficiency diseases.

56 (2016)

Empirical formulæ for the postnatal growth of the human brain and its major divisions.

By RICHARD E. SCAMMON and HALBERT L. DUNN.

[*From the Department of Anatomy, University of Minnesota, Minneapolis, Minnesota.*]

Although several graphs have been published illustrating the post-natal growth of the human brain, as well as a few of the

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[1] For review of the literature see article ''The so-called hibernating gland'' by the writer in a forthcoming number of the *Journal of Morphology.*

growth of the major divisions of the structure, apparently no attempt has been made to analyze these curves and to develop formulæ for the expression of the relation between brain weight and age between birth and maturity. We have made a series of calculations of this type and have computed empirical formulæ for the growth of the encephalon as a whole, the cerebrum, the cerebellum, and the pons, medulla and mid brain, from birth to 20 years. These formulæ have been determined from the weighted average of male and female brain weights. While it will no doubt be possible to develop slight varients of these formulæ for the weight of the entire brain and of the cerebrum for males and females separately, our data indicate that it is hardly practicable to establish separate curves for the sexes, on the basis of the material now available, for the weight of the cerebellum and the brain stem. Likewise no attempt has been made to correct graphically or mathematically for the effect of disease on the weight of the brain although all records of cases involving any brain pathology were rigidly excluded. The curves and formulæ, therefore, represent the growth of the organ in a hospital rather than in the general population.

When plotted against age and tested graphically all the curves of the postnatal growth of the brain approach hyperbolæ and may be expressed approximately by the general formulæ:

$$Y = \frac{x + c}{a + bx} \text{ or } Y = \frac{x}{a + bx} + c.$$

In these formulæ, Y is the weight of the brain or brain-part, X is the age in years and a, b and c are empirically determined constants.

A total of 2956 observations on the weight of the brain as a whole in the postnatal developmental period were available for study. Of these 317 were of newborn infants and 2639 were of individuals between birth and 20 years. The empirical formula of the curve for these data as drawn by inspection is:

$$0.01 \text{ Encephalon weight (gm.)} = \frac{\text{Age (yrs.)} + 0.315}{0.09 + 0.0692 \text{ age (yrs.)}}$$

The average deviation of the calculated values as determined by this formula from the observed trimester averages for the first year and the observed yearly averages thereafter to 20 years is 18.0 grams, and the average percentage deviation for the same values is 1.75 per cent.

The inspected curve of the postnatal growth in weight of the cerebrum, (1032 cases—108 newborn and 924 from birth to 20 years) is expressed by the formula:

$$0.01 \text{ Cerebrum weight (gm.)} = \frac{\text{Age (yrs.)} + 0.39}{0.12 + 0.00775 \text{ age (yrs.)}}$$

Encephalon

Cerebrum

Empirical Formulae:

$$0.01 \text{ Encephalon weight (gm.)} = \frac{\text{Age (yrs.)} + 0.315}{0.09 + 0.0692 \text{ Age (yrs.)}}$$

$$0.01 \text{ Cerebrum weight (gm.)} = \frac{\text{Age (yrs.)} + 0.39}{0.12 + 0.00775 \text{ Age (yrs.)}}$$

Cerebellum

Empirical Formula:

$$\text{Cerebellum weight (gm.)} = \frac{\text{Age (yrs.)}}{0.094 + 0.0078 \text{ Age (yrs.)}} + 19.8$$

Pons, Medulla and Midbrain

Empirical Formula:

$$\text{Pons, medulla and midbrain weight (gm.)} = \frac{\text{Age (yrs.)}}{0.117 + 0.041 \text{ Age (yrs.)}} + 5.0$$
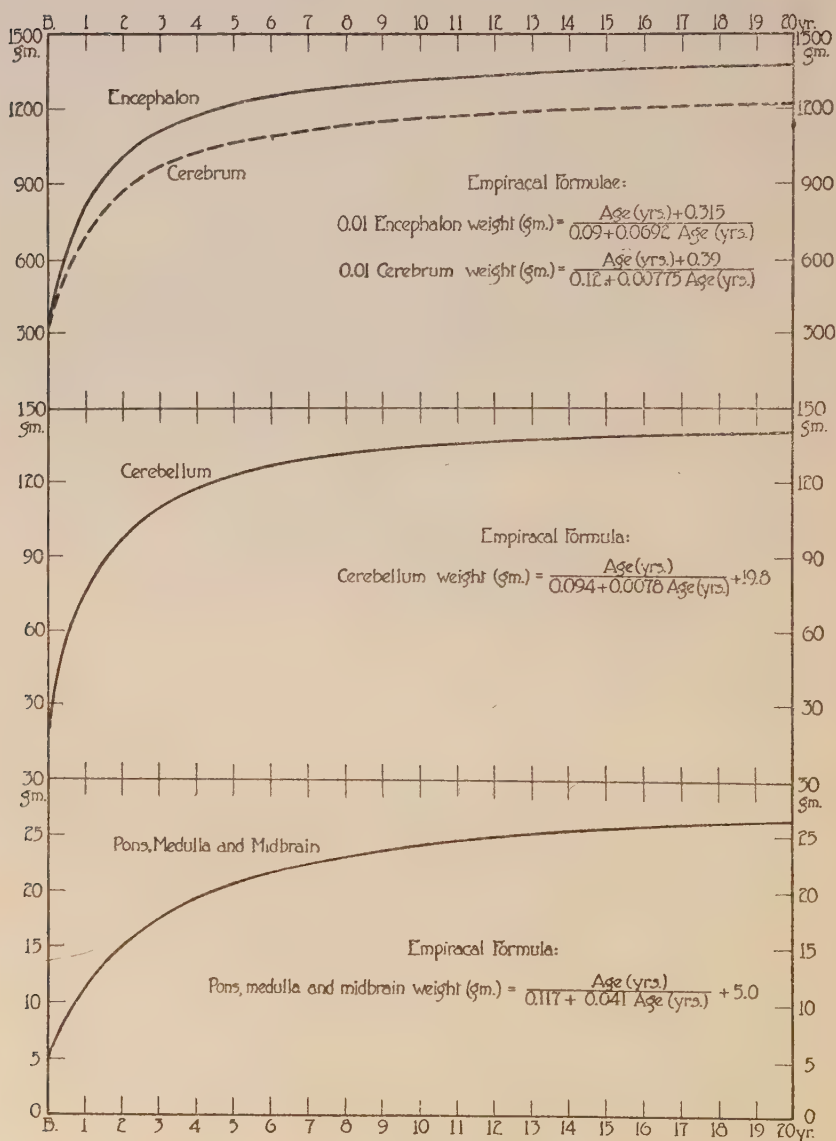
The mean deviation of the calculated from the observed averages for the time intervals as given above is 28.4 grams, and the mean percentage deviation is 2.68 per cent.

The weight of the cerebellum from birth to 20 years (890 cases—99 newborn and 791 between birth and 20 years) may be represented by the formula:

$$\text{Cerebellum weight (gm.)} = \frac{\text{Age (yrs.)}}{0.094 + 0.0078 \text{ age (yrs.)}} + 19.8$$

The mean deviation of the calculated from the observed averages is 3.82 grams and the percentage deviation is 3.72.

The empirical formula for the inspected weight of the pons, medulla and midbrain (857 cases—100 newborn and 757 between birth and 20 years) is:

$$\text{Pons, medulla and midbrain weight (gm.)} = \frac{\text{Age (yrs.)}}{0.117 + 0.041 \text{ age (yrs.)}} + 5.0$$

The mean deviation of the calculated values from the observed averages in 0.25 gm. and the mean percentage deviation is 2.2 per cent.

The accompanying graph illustrates the curves of postnatal growth of the brain and its parts as drawn to the formulæ given above.

ABSTRACT OF COMMUNICATIONS

Seventh meeting.

Minneapolis, Minnesota, November 8, 1922.

57 (2017)

Biochemical properties of the blood of pigeons in polyneuritis and starvation.[1]

By LEROY S. PALMER and CLARA T. HOFFMAN (by invitation).

[*From the Section of Animal Nutrition, Division of Agricultural Biochemistry, University of Minnesota, St. Paul, Minnesota.*]

The data in Table 1, showing the analyses of the whole blood of (1) normal pigeons, (2) normal pigeons starved until they had lost 40 per cent. of their body weight, (3) pigeons with "latent" polyneuritis, (4) pigeons with acute polyneuritis, (5) pigeons which had attained normal weight following acute polyneuritis, *i.e.* "relieved polyneuritis," and (6) pigeons starved to a 40 per cent. loss of weight following "relieved polyneuritis," indicate that the decrease in total solids and in the nitrogenous constituents of the blood, both protein and non-protein, as well as the diminution in the erythrocytes, are probably due to the self-imposed starvation which accompanies the later stages of polyneuritis in pigeons. It is to be noted, however, that simple inanition in its advanced stages is accompanied by a decline in the relative amount of protein in the blood which is not apparent even in acute polyneuritis. This result is contrary to the recent observations of Pacchinsa.[2]

None of the usual "symptoms" of polyneuritis accompanied simple inanition in these experiments. The polyneuritis diet consisted of a mixture of polished rice, casein, butter fat and salts.

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[1] This problem was suggested in part by R. A. Dutcher, formerly of the University of Minnesota. Credit is also due Professor Dutcher for assistance rendered in the early phases of the work.

[2] Pacchinsa, S., *Ann. di clin. med.*, 1921, ll, 271.

TABLE I.   AVERAGE ANALYSES OF PIGEON'S BLOOD IN POLY-NEURITIS AND STARVATION

State of nutrition	Number of birds	Total solids	Total Nitrogen	Protein Nitrogen	Protein nitrogen in total solids	Non-protein nitrogen	CO_2 in 100 c.c. plasma	Total Erythrocytes per cm. $\times 10^6$	Total Leucocytes per cm. $\times 10^5$
		per cent.	per cent.	per cent.	per cent.	per cent.	c.c.		
Normal	13	22.24	3.46	3.305	14.87	0.155	49.6	3.1	1.7
Normal starved	8	17.83	2.66	2.561	14.35	0.097	44.2	2.8	2.8
Latent polyneuritis	7	18.73	2.88	2.829	15.10	0.051	57.5	2.5	2.2
Acute polyneuritis	16	16.65	2.55	2.483	14.95	0.065	49.9	2.2	2.6
"Relieved" polyneuritis	4	22.59	3.46	3.381	14.99	0.079	61.3	3.4	3.9
"Relieved" polyneuritis starved	3	18.46	2.42	2.323	12.60	0.097	50.3	3.2	3.0

58 (2018)

Protein content of frog's plasma.

By R. A. BIETER and F. H. SCOTT.

[*From the Department of Physiology, University of Minnesota, Minneapolis, Minnesota.*]

In the *Journal of Experimental Pathology* of October, 1921, Hill and McQueen made some observations on capillary pressure of the frog's kidney, which they believe indicate that filtration is not possible. They found capillary pressure of about 10 millimeters, while the pressure in the arterioles was from 25 to 30 millimeters. A pressure of 10 millimeters might still be large enough to produce filtration if the colloid content of the frog's blood was low enough. We have accordingly made some determinations on the protein content of the frog's plasma. This was done by drawing blood from the heart of frogs into tubes containing finely divided potassium oxalates. The blood was then centrifuged and the plasma obtained. Kjeldahl determinations were done on the plasma and reckoning the total nitrogen found as all protein, and using the factor 6.25, the protein of the frog's blood is between 0.6 and 0.8 per cent.—approximately 10 per cent. of that of mammals. With this small colloidal content it is evident that very few millimeters of mercury would be sufficient to permit filtration. Thus the low capillary pressures observed by Hill and McQueen are no arguments against filtration.

It may be noted that Halliburton[1] gives the content of frog's plasma at about 2.5 per cent. protein. Our results were obtained from frogs in the late fall, and whether Halliburton's results are due to a seasonal variation or whether it is a difference of method we do not know, as Halliburton does not give details as to how he obtained his plasma.

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[1] *Journal of Physiology*, 1886, vii, 319.

59 (2019)

Function of the precentral convolution in primates.

By K. S. LASHLEY.

[*From the Psychological Laboratory, University of Minnesota, Minneapolis, Minnesota.*]

Studies of cerebral function in rodents have shown the survival of habitual reactions after bilateral destruction of the electro-stimulable pallium and of the corpora striata.[1] The present experiments were designed to test the relations of the corresponding cortical areas in primates to the performance of habitual or voluntary movements.

Two Cebus monkeys were trained in manipulative movements of the hands; opening various types of latch boxes. When facility in these movements was acquired, the animals were subjected to an operation in which all of the electro-stimulable cortex anterior to the Rolandic fissure on each side was destroyed by cauterization. The animals were then kept without additional training until they recovered from the resultant paralysis. When recovery was sufficient to permit of grasping movements, retention of the latch-box habit was tested. Both animals showed perfect retention of the habits, reproducing the specific manipulative movements acquired through the initial training.

The experiment seems to prove that destruction of the so-called motor area of the pallium does not destroy the specific patterns of neural integration involved in habitual or voluntary movements and hence, that the motor area is not the chief normal efferent path from the cortex for voluntary movement.

On the basis of earlier work with rodents the suggestion is advanced that the motor pallium is to be considered a relatively primitive center for regulation of postural reflexes, facilitating the neural impulses for voluntary movement, but taking no direct part in their integration or transmission from the cortex.

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[1] Lashley, K. S., *Brain*, 1921, xliv.

SCIENTIFIC PROCEEDINGS

60 (2020)

Is Bacillus Acidophilus therapy a strictly bacteriological phenomenon?

By NICHOLAS KOPELOFF.

[*From the Department of Bacteriology, Psychiatric Institute, Ward's Island, New York City.*]

Having obtained relief from chronic constipation and diarrhea by treatment with milk fermented with *B. Acidophilus,* further studies are in progress to determine whether the essential nature of this phenomenon is physical, chemical or bacteriological.

Subject to the limitations of the material under consideration the following points have been established:

1. *B. Acidophilus* therapy is not a physical phenomenon since patients receiving sterile milk were not relieved of constipation.

2. *B. Acidophiius* therapy apparently is not a strictly chemical phenomenon, since patients receiving *B. Acidophiius* milk which had been pasteurized to kill all living forms, were not relieved of constipation.

3. *B. Acidophilus* therapy appears to be essentially a bacterio-

logical phenomenon, since patients were relieved of constipation by the ingestion of milk fermented by *B. Acidophilus*.

4. Relief from chronic constipation has persisted for six months after the ingestion of *B. Acidophilus* has been discontinued.

5. Viable *B. Acidophilus* organisms in appreciable number have been recovered from the feces of patients six months after the ingestion of *B. Acidophilus* milk.

61 (2021)

Brom cresol green, a sulfonphthalein substitute for methyl red.

By BARNETT COHEN.

[*From the Hygienic Laboratory, Washington, D. C.*]

The sulfonphthalein indicators of Clark and Lubs have shown themselves quite stable and reliable in biological fluids. Methyl red, which is not a sulfonphthalein, is not altogether reliable, but was included in the Clark and Lubs series because it was indispensable in covering a certain range of H-ion concentration. Methyl red is easily reduced irreversibly to a colorless compound—frequently by microbic action—thereby impairing its utility as an indicator under all conditions.

A sulfonphthalein indicator has been synthesized which has an apparent dissociation constant almost identical with that of methyl red and which seems as stable and reliable as the rest of the sulfonphthaleins. This compound is tetra-brom m-cresol sulfonphthalein. It is made by the bromination in glacial acetic acid of m-cresol sulfonphthalein. The common name suggested for this compound is *Brom Cresol Green*. Its effective range as an acid-base indicator is between P_H 4.0 and 6.0, with a color change from yellow to green to blue-green. Its apparent dissociation constant in terms of P_H is 5.00 (that of methyl red is 4.95).

Color standards of *Brom Cresol Green* were unaffected after several months' exposure in test tubes to usual laboratory conditions, while similar standards of methyl red had faded irregularly and become totally useless. *Brom Cresol Green* may be used directly in a bacteriological culture medium, for instance, while this would be out of the question for methyl red.

62 (2022)

Can fasting fowls synthesize glycocoll or ornithine?

By JESSE G. M. BULLOWA and CARL P. SHERWIN.

[*From Fordham University, Research Laboratory, New York City*]

According to Suga[1] "starving hens (unlike well-fed birds) are unable to synthesize hippuric acid from benzoic acid and glycocoll; they conjugate injested benzoic acid with ornithine into ornithuric acid and excrete the latter compound." This is contrary to other authors, as Thomas[2] found that chickens kept on an inadequate diet could not even produce ornithine for conjugation with benzoic acid. Crowdle aand Sherwin[3] found that hens on a carbohydrate diet were able to synthesize ornithine under these conditions but found no glycocoll compounds; in other studies[4] where chickens were fed toxic organic compounds such as were detoxicated in the animal body by union with glycocoll, no combination with glycocoll was ever found. They concluded that glycocoll was never used by the chicken for detoxication purposes and these results were corroborated by the work of Yoshikawa[5] who fed chickens benzoic acid and glycocoll

1 Suga, T., *Kyoto Igaku Zasshi*, 1919, xv, 225; *Jap. Med. Lit.*, 1920, v, 46; *Chem. Abstracts*, 1921, xv. 881.

2 Thomas, K., *Centralblatt f. Physiol.*, 1914, xxviii, 769.

3 Crowdle, J. H., and Sherwin, C. P. Results unpublished.

4 Sherwin, Carl P., and Crowdle, Jas. H., Proc. Soc. Exp. Biol. and Med., 1922, xix, 318.

5 Yoshikawa, J., *Zeit. Physiol. Chem.*, 1910, lxviii, 79.

together and found that, in spite of this, benzoic acid was not conjugated with glycocoll but that it was burned in the body of the fowl and the benzoic acid conjugated with ornithine. Unfortunately this experimenter does not state whether his birds were "well fed" nor does he even mention the type of diet. It seemed worth while to follow up this clue regarding glycocoll synthesis in the organism of the fowl and at the same time we thought it might give some valuable hints as to the difference between the metabolism during fasting and while on a normal diet. We decided to place a few hens on a normal complete diet and then feed them benzoic acid in order to determine whether glycocoll might conjugate with the benzoic acid, thus causing the excretion of hippuric acid and then after investigating this point to take up the point of difference between Thomas and Suga regarding the power of the starving hen to furnish ornithine for the detoxication of benzoic acid.

In our work hens were provided with an artificial anus according to the method of Voeltz.[6] It was found unnecessary to provide the chicken with two different receptacles for urine and feces, as the chickens were seldom able to defecate and had to be given enemas both morning and evening. The urine, on the contrary, while usually a white pasty mass adhering to the feces, was excreted in considerable volume—ranging from 200 to 500 c.c. per day. The urine in this case consisted of a white muddy precipitate (uric acid) and a serous-like liquid. The hens were placed in a small metabolism cage provided with a coarse wire screen flooring and drain. The cage was washed at the end of each forty-eight hours with an 0.05 normal solution of sodium hydroxide to remove any traces of organic acids and to wash off, but not dissolve, the uric acid clinging to the parts of the cage. The diluted alkaline urine was then filtered by suction, exactly neutralized and evaporated to dryness at 40° with the aid of an eleectric fan.

It is claimed that the intestinal tract of the chicken may be completely evacuated in the course of six hours; we, however, found that from the nitrogen determinations made on the urine that only after two days of fasting had a stage of endogenous nitrogen metabolism been reached.

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6 Voeltz, W., *Handbuch d. Biolog. Arbeitsmethoden*, 1922, Abt. IV, Teil 9, Heft II, 300.

At this stage we started feeding benzoic acid and gave each of the three hens one gram of benzoic acid per day for five days, during which time the hens received no food but plenty of water. The evaporated urines were united, acidified with phosphoric acid and extracted with alcohol in a rotatory extracting apparatus. This alcoholic extract was then evaporated to dryness by means of an electric fan and shaken several times with large quantities of ether. The ether extract was allowed to stand in the ice-box for nearly a month, then as no benzoyl-ornithine crystallized out, the ether was distilled off and the brown residue dissolved in hot water. On cooling, crystals appeared in the water solution which resembled those of benzoic acid. The crystals were removed by suction and recrystallized from hot water.

On drying the crystals melted at 120-121°, proving the substance to be unchanged benzoic acid. The amount of benzoic acid thus recovered amounted to 9.5 grams or 63.3 per cent. of the amount fed. The residue from the original alcoholic solution was again extracted but this time with a mixture of seven parts ether and three parts alcohol at 30°. This extract was placed in a stoppered flask and kept at 0° and each day sufficient ether added to cut down the percentage of alcohol one per cent. When the percentage of alcohol had been reduced to three per cent., there appeared a granular white precipitate on the bottom of the flask. This substance was redissolved in alcohol and precipitated by the addition of ether, dried at 80° in vacuo and found to melt at 180-184°, but the total yield of the pure substance was slightly more than 0.1 grams. Nitrogen determination by Kjeldahl showed 8.06 per cent. instead of the calculated 8.23 per cent.

"Well fed" hens were also fed 15 grams of benzoic acid, that is, three different hens were given one gram of benzoic acid per day for each of five days. The urines were treated in exactly the same manner as described above.

As a result of this feeding we isolated 3.1 grams of benzoyl-ornithine from the urine of the hens and 8.3 grams of uncombined benzoic acid. Inasmuch as we found no trace of a glycocoll compound it seemed that perhaps hippuric acid had been excreted but had escaped detection. For this reason a hen was fed one gram hippuric acid per day for a period of three

days. The urines were evaporated and extracted with the same alcohol ether mixture. From this extract or from the water solution of the residue of this extract, we recovered 2.3 grams of the ingested hippuric acid without difficulty.

It would seem then that starving hens do furnish a very small amount of ornithine when this is necessary for the detoxication of benzoic acid. Contrary to the claims of Suga however, we were unable to find even a trace of hippuric acid in the urine of well fed birds after the feeding of benzoic acid but instead only benzyl ornithine or free benzoic acid and like Yoshikawa we believe that birds are unable to furnish glycocoll for detoxication purposes and even unable to make use of it, if it is furnished them from exogenous sources.

63 (2023)

The experimental production of gall-stones in dogs, in the absence of infection, stasis, and gall bladder influence upon the bile.

By PEYTON ROUS, P. D. McMASTER, and G. O. BROUN.

[*From the Rockefeller Institute for Medical Research, New York City.*]

Numerous circumstances and influences which favor the development of gall-stones are now recognized, but uncertainty exists as to which of them are contributory in character and which critical, and as to whether indeed the decisive causes for cholelithiasis are to be found amongst them. In this connection, observations under controlled conditions in animals possess interest.

By a method elsewhere reported,[1] it is possible to join a rubber tube to the common duct of a dog and collect the bile under sterile conditions for months. The gall bladder should be removed at the time of intubation. Our animals thus treated remained in

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[1] Rous, Peyton, and McMaster, P. D., *Jour. Exp. Med.*, 1923, xxxvii, 11.

excellent condition, but the observations on several of them were cut short by calculus formation in the collecting system. Twelve dogs have been studied with relation to this development. Calculi were found in six, and in three of these the bile had been sterile. In two of the three instances, the calculi gradually filled the 2 mm. lumen of a glass canula on the wall of which they were sessile, and gave rise to obstruction. Once this happened within twenty-one days of intubation.

The calculi were found only on the walls of the collecting system of rubber and glass, never in the ducts themselves; and they occurred in none of five instances in which this system remained clear of organic débris (dead cells and mucinous matter), but in six out of seven in which there was lodgment of such material,—from which the ducts were always practically free. The stones were multiple, discrete,—at least to begin with,—of approximately the same size at any given level in the tube system, but larger toward the glass canula inserted into the common duct, and more numerous and larger on the lower side of the tube lumen and wherever there existed the possibility for an eddy in the bile current or a dead space, as where glass and rubber joined. The calculi that had formed on the glass connections could be examined directly with the microscope. Early stages were studied in this way.

The stones were made up of calcium bilirubinate and calcium carbonate, with a scaffolding of organic material. Cholesterol was not demonstrable in them. The majority had a center of calcium bilirubinate surrounded by an envelope of crystalline, slightly pigmented carbonate; but stones consisting almost wholly of one or the other substance were encountered. Frequently a number of pigment stones were secondarily united in a matrix of carbonate. The relation of the calculi to the organic débris associated with them differed significantly. Those formed primarily out of carbonate originated in the midst of lumps of the débris, as the microscope showed, whereas the minute pigment stones were so situated as to suggest that they, or their original nuclei, had once been free in the bile but had been caught in the débris and retained. Some of the pigmented calculi were large and of such shape as to leave no doubt that deposition had occurred upon them *in situ*.

The relation between calcium bilirubinate and calcium car-

bonate in the stones of the dog has similarities to that between calcium bilirubinate and cholesterol in human calculi. There is the same tendency in both instances for a nucleus of the pigment material to be overlaid with another substance.

The observations here reported show that infection is not the essential factor in cholelithiasis. That it frequently plays the determining rôle is equally certain.[2] This it would seem to do by damaging the duct wall with result in desquamation,—which the sterile canula did in our experiments,—and by lessening the ability of the bile channels to rid themselves of the cell débris. The débris induces, or furthers, the direct deposition of solids, and may catch and retain potential nuclei for stone formation, in the shape of pigment particles from higher up in the biliary system, which would under ordinary circumstances be voided with the bile.

64 (2024)

The paradoxical shortening of blood coagulation after intravenous administration of sodium citrate.

By NATHAN ROSENTHAL and GEORGE BAEHR.

[*From the Department of Pathology and the First Medical Service, Mount Sinai Hospital, New York City.*]

Sodium citrate, when administered intravenously in large doses (0.5 grams for dog or cat, 3.0 to 6.0 grams for man), produces a pronounced and progressive shortening in coagulation time of the blood which usually reaches its maximum within one hour and may persist for many hours. As a rule, the coagulation time slowly returns to normal within twenty-four hours.

This action of sodium citrate upon the coagulation of the blood *in vivo* is exactly opposite to what occurs *in vitro*. We

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[2] Gilbert, A., et Fournier, L., *Sc. et mém. de la Soc. de Biol.*, 1897, iv, 10 Ser., 936; Mignot, R., *Arch. gen. de Med.*, 1898, i, 129.

believe that it is dependent upon some effect on the blood platelets, which are not directly destroyed by the citrate but are damaged by contact with it and are then removed from the circulation by the spleen where they are destroyed and their thromboplastic contents gradually liberated into the circulating blood.   This theory is based upon the following observations:

1. In the test tube, sodium citrate does not destroy the platelets, but it effects them so that they are actually preserved and therefore more easily counted.

2. Within a few minutes after the intravenous injection of sodium citrate the blood platelets often begin to diminish in number, the maximum reduction being usually observed after ten to fifteen minutes and the number as a rule returning to normal within half to one hour.   The greatest reduction in blood platelets was observed in cats, in two of which 85 per cent. and 90 per cent. of the platelets disappeared from the circulating blood within ten and fifteen minutes respectively, and the count again reached normal a half hour after the injection.

3. Increasing amounts of free thromboplastic substance (cytozyme) probably derived from platelets begin to appear in the blood stream as the coagulation time becomes shortened.

4. No changes in the content of the blood in the other factors concerned in coagulation, such as calcium, fibrinogen or antithrombin, are demonstrable.

5.   The increase in the thromboplastic agent cytozyme and the shortening in coagulation time of the blood do not occur simultaneous with the numerical change in the platelets, but follow it. The maximum shortening in coagulation time occurs some time after the numbers of platelets have again returned to normal, and persists for hours.

6. The characteristic shortening of coagulation time after intravenous injection of sodium citrate does not occur in animals (ducks) in whose blood few or no platelets occur.   In fact, if sufficient citrate is administered, the opposite effect is accomplished in such animals and the coagulation time becomes markedly prolonged.

7. The shortening of coagulation time after intravenous injection of sodium citrate likewise fails to occur in human beings suffering from hemorrhagic blood diseases, in whose

blood there is also a pronounced numerical deficiency in blood platelets.   In purpura hemorrhagica, in which such a reduction in the number of platelets is regularly present, the injection of sodium citrate may be followed by almost complete disappearance of platelets from the circulating blood.   In both these diseases and in congenital hemophilia, in which there is presumed to be some deficiency in the quality of blood platelets, the injection of sodium citrate is followed by a prolongation in the coagulation time, a further diminution of the blood platelets and by a marked increase in the bleeding tendency.

8. The characteristic shortening of coagulation time does follow intravenous administration of sodium citrate in cases of obstructive jaundice, in which, although the coagulation time is prolonged and there is a bleeding tendency; the blood platelets in this condition are normal in number and unaffected.

Sufficient evidence has therefore been presented to indicate that the shortening of coagulation time after injection of sodium citrate in normal individuals or animals is due to some influence on the blood platelets.   That this is not a direct destruction is indicated by the test tube experiment and by the fact that the maximum shortening of coagulation time does not occur simultaneous with the maximum reduction in platelets, but follows at some time between ten minutes to one hour later.

This sequence suggests that the platelets after contact with the citrate are damaged and removed from the circulation by some organ, there destroyed and their thromboplastic contents gradually liberated into the blood stream.   The removal and destruction of damaged blood platelets is probably one of the functions of the spleen, especially in view of the rôle which it is presumed to play in the thrombopenia of purpura hemorrhagica.

Based upon these observations the *slow* intravenous injection of large doses of sodium citrate up to five grams has been successfully employed in arresting internal hemorrhages due to gastric ulcer, typhoid fever, pulmonary tuberculosis, etc., and also as a pre-operative prophylactic measure in obstructive jaundice.   In hemorrhagic blood diseases for reasons detailed above, its use is strictly contraindicated.

65 (2025)

Distribution of phosphorus in the blood.

By T. F. ZUCKER and MARGARET GUTMAN.

[*From the Department of Pathology, College of Physicians and Surgeons, Columbia University, New York City.*]

It is well known that when blood is allowed to stand after it has been drawn, its content of inorganic phosphate will gradually increase to 1 mgm. or more above the figure which is obtained when the determination is carried out immediately. This indicates that the organic substances containing phosphoric acid which occur in the blood, particularly in the red cells, in connection with which Greenwald has introduced the name "acid soluble phosphorus" are slowly hydrolyzed to yield inorganic phosphate. This hydrolysis, however, is incomplete even when aided by boiling with dilute acid. This finding would seem to indicate that there must be two types of organic phosphoric acid combination in the blood,—one of which is easily hydrolyzed and the other one not. To investigate this further, we have carried out phosphorus determinations by Tisdall's micromethod on the protein-free filtrate of blood, obtained by means of trichloracetic acid. We determined the inorganic phosphate immediately and again after boiling the filtrate for two hours. The total phosphorus in the filtrate (acid soluble phosphorus) was also determined. The results are shown in the accompanying chart. This shows that a constant amount of phosphoric acid is split off by boiling, approximately 10 mgm. of phosphorus being thus obtained in human blood and 13 mgm. in rat's blood. This then leaves for the organic hydrolysable phosphoric acid in the human 6-7 mg. and in the rat 5-7 mg. If we subtract the figure obtained after boiling from the total acid soluble, we obtain the figure representing the amount of phosphoric acid contained in organic combination which is not hydrolysed under the conditions stated. We have, therefore, evidence of three forms of phosphoric acid which will pass into aqueous filtrates on coagulation of the protein.

It is evident that the above needs further substantiation. In the first place, we must give evidence that the determination of

inorganic phosphate really represents all inorganic phosphate present, for it might be thought that the above results are merely a simulation of what we claim, due to limitations of the method used. We, therefore, added to a sample of blood, sodium phosphate equivalent to 1 mgm. of P per 100 c.c. of blood. The table shows the results. We find a complete recovery both in the inorganic phosphate and the determinations after boiling within the limit of error of the method.

To demonstrate the completeness of hydrolysis of the hydrolysable substance, we have done further experiments in which hydrochloric acid or nitric acid were added to the filtrate before boiling. The trichloracetic acid is probably all decomposed before the end of the two hours boiling. Either of these acids when added in amounts to produce a concentration of about N/4 will somewhat interfere with an accurate determination by the Tisdall method, so that after two hours boiling lower results are obtained. However, when we continued the boiling for four hours and then applied the Tisdall method, the results were identical with those obtained on boiling with only trichloracetic acid present in the filtrate. (11.1 mgm. with HNO_3 against 11.3 without HNO_3 on beef blood.)

It might still be objected that the micromethod is not applicable. We have, therefore, repeated the experiments on a larger scale, with 200 c.c. of blood filtrate using for the determinations of phosphorus the well-recognized method of preliminary precipitation with ammonium molybdate, precipitation and reprecipitation with magnesia mixture, ending with gravimetric determinations as magnesium pyrophosphate. The small amount of phosphorus which results even from the 200 c.c. of filtrate does not allow an accurate quantitative determination, but it clearly shows that boiling with acid yields a relatively constant figure distinctly higher than the inorganic and distinctly not equal to the total phosphorus of the filtrate.

The only objection still remaining, is the possibility of incomplete precipitation in blood filtrates by all molybdic acid reagents and a partial removal of an inhibiting substance by boiling with acid. A determination of the phosphate obtained by precipitating the blood filtrate directly with magnesia mixture (heated, and allowed to stand over night) gives values which are incorrect, due to impurities in the precipitate. We have, however,

DISTRIBUTION OF PHOSPHORIC ACID IN BLOOD

	Inorganic mg.	After boiling mg.	Total acid soluble mg.	Total Organic		Organic hydrolysable		Organic non-hydrolysable		Remarks
				mg.	Per cent.	mg.	Per cent.	mg.	Per cent.	
Human............	2.65	9.40	19.3	16.65	86.4	6.75	34.9	9.9	51.3	March
	2.80	10.50	20.0	17.2	86.0	7.70	38.0	9.5	47.5	June
	2.55	9.30	19.0	16.45	86.8	6.75	35.3	9.7	51.0	December
Rat, normal......	7.6	13.5	19.5	11.9	61.1	5.9	30.2	6.0	30.8	Flour diet containing 20 per cent. dry milk.
	6.66	13.4	20.0	13.4	67.0	6.74	33.7	6.6	33.0	Flour diet containing 20 per cent. dry milk and 2 per cent. Na_2CO_3
	5.4	13.0	20.6	15.2	73.8	7.6	36.9	7.6	37.0	Flour diet with 5 per cent. dry milk.

RECOVERY OF ADDED PHOSPHATE

	Inorganic mg.	After boiling mg.
Blood, human ..	2.65	11.05
	2.76	11.1
		11.2
Same blood + 1 mg. P per 100 c.c.	3.60	12.1
	3.56	12.3
		12.05

determined that, after this procedure which causes hydrolysis, a considerable quantity of phosphorus is present in the filtrate. This amount agrees closely with that of the fraction which we have termed "non-hydrolysable phosphate."

66 (2026)

Further observations on the chemistry of cod liver oil.

By T. F. ZUCKER.

[*From the Department of Pathology, College of Physicans and Surgeons, Columbia University, New York City.*]

It has been stated previously[1] that the constituent of cod liver oil which influences the mineral metabolism, when cod liver oil is used in treating rickets, is contained in the unsaponifiable fraction of the oil. Further attempts towards isolating the active material have yielded the following results. A good yield of crude product can be obtained by directly extracting cod liver oil with 95 per cent. alcohol. This mixture of fatty acids, a small amount of oil, and other substances, is saponified with sodium hydroxide, and when the calcium soaps are precipitated from an aqueous solution, the unsaponifiable material including the active substance, is precipitated with the soap. From this calcium soap, acetone will extract the active material. In this manner we have obtained preparations of the active material which after a dilution of 1 :1000, are as active as the original cod liver oil. The chemical nature of the substance has not yet been determined, but we believe that we are approaching its actual isolation.

With regard to the properties of the active material thus obtained, we can say that it is not toxic in doses of more than 50 times the curative dose. A single large dose in our experiments brought about healing at the same rate as a succession of small doses. The purified active material is entirely free from fat soluble A as shown by the fact that it will not cure xerophthalmia when a subsequent treatment with butter fat does cure the condition.

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[1] Zucker, Pappenheimer and Barnett, PROC. SOC. EXP. BIOL. AND MED., 1922, xix, 167.

67 (2027)

A note on the entrance of the spermatozoon into the starfish egg.

By ROBERT CHAMBERS.

[*From the Department of Anatomy, Cornell University Medical College, New York City.*]

In 1876 Fol made the classic discovery that the spermatozoon actually enters the egg in fertilization. This fact he observed in the starfish egg. Fol's treatise was apparently so exhaustive and so carefully worked out that no one has questioned the details of his observations and his interpretation of the process is generally accepted to this day. Conical elevations were seen to form on the surface of the egg and the spermatozoa travelled in a straight line toward them. When a spermatozoon reached a cone its head penetrated it. Fol called the conical elevation the "attraction cone" and believed that it attracted the spermatozoon from a distance.

The starfish egg is surrounded by a zone of glutinous jelly the thickness of which is about one fifth the diameter of the egg. When the eggs are placed in a sperm suspension all the spermatozoa that accidentally come into contact with the surface of the jelly stick and are unable to penetrate it to any extent.

My observations confirm those of Fol regarding the formation of the cones on the egg's surface. The number of cones depends upon the age of the egg and upon the density of the sperm suspension surrounding it. An overripe egg forms these cones quickly and in considerable numbers. A fresh mature egg forms only a few cones unless the sperm suspension is very dense.

Fol, however, failed to observe the following: From the tip of each cone a slender filament grows outward piercing the jelly until it reaches the periphery where the trapped spermatozoa are lying. If there be no spermatozoa in the immediate vicinity nothing more happens. If, however, the tip of the filament comes into contact with a spermatozoon the cytoplasm of the tip and that of the sperm head immediately flow together so that the sperm nucleus now lies within the cytoplasm of the egg fila-

ment.   An extraordinary reaction then takes place.   The fila-
ment begins to draw back into the egg dragging the spermato-
zoon along with it.   Not only this but all the other filaments
projecting from the egg are similarly withdrawn.   Apparently,
a wave of response is started when a filament fuses with a sper-
matozoon.   This wave must travel down the filament and over
the egg.

As the filament with a spermatozoon on its tip shortens, the
spermatozoon is pulled deeper and deeper into the jelly and the
lashing of its tail becomes more and more restricted.   The sper-
matozoon behaves like an unwilling victim and occasionally,
frees itself, especially when other filaments have been slightly
ahead in activity and have also secured spermatozoa which they
are now pulling in.   With the microdissection needle one may
free a spermatozoon by breaking the filament to which it is at-
tached.   Such a spermatozoon is generally unable to extricate
itself from the jelly in which it lies embedded.   After a few vi-
brations of its tail it becomes permanently quiescent.

By the time the filament has dragged the spermatozoon half
way through the jelly the base of the cone changes in shape.
The convexly rounded border, which gives it the appearance of
a rounded nipple, draws in so as to become concave.   In doing
so it leaves the egg membrane behind and this now becomes
plainly visible owing to the space intervening between it and the
surface of the cone.   By the time the filament is withdrawn so
as to bring the sperm head to the summit of the cone, the lifting
of the egg membrane has spread from the base of the cone over
the egg and is recognized as the fertilization membrane.

When the filament is completely withdrawn into the base of
the cone the head of the spermatozoon is taken in with it.   The
tail of the spermatozoon remains for a time outside the fertiliza-
tion membrane.   As long as the tail maintains organic continu-
ity with its head it keeps up a feeble oscillatory movement.   As
the cone recedes into the egg, the strand extending from it to
the tail outside the fertilization membrane breaks and the tail then
lies motionless.   The tail can be seen for several minutes mark-
ing the site where the sperm head had gone in.

68 (2028)

The quantitative determination of the alkali retention in growth.

By ALFRED T. SHOHL, M. D.

[*From the Department of Pediatrics, Yale University School of Medicine, and the New Haven Hospital, New Haven, Conn.*]

In a report made last year[1] on acid base metabolism in infants, a method was proposed for measuring the retention or the loss of alkali in the body. The method consists in determining the equilibrium or balance of all the acid and basic radicals. The values are computed in terms of normal solutions and the excess of base retention or excretion can then be stated in terms which are a common denominator for all of the elements in mineral metabolism. It is necessary to determine the acids and bases in the food, urine and feces. The results calculated in terms of normal solutions are then totaled.

By suitable methods of titrating the urine and stools, approximately the same values are obtained as by the method of analyzing the individual elements. The work will be reported in extenso in a forthcoming number of the American Journal of the diseases of Children, including methods and protocols.

The metabolism experiments show that for an infant, A, weighing 9 kilos, and one, B, weighing 5½ kilos, fed on cow's milk, water and sugar, the positive base balance, as shown in the accompanying figure, was 98 c.c. 0.1N alkali in excess of acid and 64 c.c. 0.1N alkali in excess of acid. Calculated on the basis of alkali retained per kilogram of body weight, this equals 11 c.c. O.1N alkali retained per day in both cases.

	A	B
Intake	—255 c.c 0.1N	—211 c.c. 0.1N
Output	—157	—147
+ base balance or base retained	98	64

A recalculation of the available data in the literature confirms our results. This appears to be a very large amount of base

1 Shohl, Alfred T., Acid Base Metabolism in Infants, *Journal Biological Chemistry*, 1922, xxxvi, 50.

retention per day. However, when the values are computed it is found that of this alkali retained, 2 c.c. is required for the protein increase, 4 c.c. for the alkali reserve and about 57 c.c., by far the greatest part, for the building of bone.

The effect of acids and alkalis added to the diet has also been studied. When 250 c.c. of 0.1N HCl was given baby A he retained 67 c.c. of alkali in excess of acid. When 4 grams of sodium bicarbonate, equivalent of 473 c.c. of 0.1N alkali was given to baby B he retained 114 c.c. of base in excess of acid. This is a quantative measure of the effect of acidosis and alkalosis in relation to alkali retention and growth.

It is hoped that studies of this type now being pursued will yield further information on the problems of growth, nutrition and normal and pathological mineral metabolism.

69 (2029)

On the effect of certain drugs, notably quinine, on the acuity of hearing.

By A. G. POHLMAN and F. W. KRANZ.

[From the Department of Anatomy, St. Louis University, St. Louis, Mo., and Wallace Clement Sabin Laboratory of Acoustics, Riverbank, Geneva, Ill.]

The purpose of this investigation was to determine the degree of deafness following the administration of quinine and also to ascertain, if possible, whether or not the tinnitus which accompanies this intoxication has any appreciable effect on the acuity of hearing at any and all pitches. While these experiments were under way the writers found that Macht, Greenberg and Isaacs had published a similar investigation in reference to antipyretics. It was thought desirable, for reasons which will appear later, to check on the results obtained by these writers. The quantitative tests submitted in this paper were all made by Kranz[1] with a thermophone provided with a new frequency vari-

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1 Kranz, F. W., Phy. Review, 1921, xvii, 384

ator of his own design. The drug effects were studied both subjectively and objectively by Pohlman[2]. We are indebted to Colonel George Fabyan of Riverbank, Geneva, Ill., for the opportunity of collaborating on this problem of applied physics.

The article by Macht, Greenberg and Isaacs[3] reviews the literature thoroughly and includes certain notations on the effects of quinine of which we shall speak later. They confined their attention to antipyretics and to certain combinations of these drugs. In a general way, they exhibited small doses of the various drugs to normal individuals and after an hour interval tested the acuity of hearing. This was done by comparing the distance at which a watch tick could be heard before and one hour after the administration of the drug. The difference therefore gave a reading of greater or lesser distance. The authors state that the figures are after all only empirical guides because they recognize "true intensity varies as the square of the distance of the sounding object."

Inasmuch as this paper is mainly directed toward suggesting more definite methods, it may be well to point out certain disadvantages in the use of the watch as a test for minimum audibility. The watch tick is not only a difficult sound, or rather series of sounds, to handle but the conditions under which the usual tests with a watch are taken make quantitative measurements practically valueless for anything approaching accuracy in results. This statement must not be construed as a specific criticism. It is to be lamented that no better quantitative test methods were available for the work on the antipyretics. This same objection holds for the exhaustive study by Kato[4] on the reflex responses of the M. tensor and M. stapedius in experimental animals even when the various pitches used were generated by a Galton whistle actuated by a definitely controlled air pressure. The distance at which a given watch tick will be heard is not only dependent on the character of the watch and the manner in which it is held but it is also dependent on the standing wave system in the room in which the test is being conducted. This is never the same in any two rooms or is it the same in any

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2 Pohlman, A. G., *Annals of Ot., Rhin. and Lary.*, 1922, xxxi, 1 and 430.

3 Macht, Greenberg and Isaacs, *Jr. Phar. and Exp. Med.*, 1920, xv, 149.

4 Kato, *Arch. and Ges. Physiol.*, 1913, cl, 569.

two spots in the same room. The statement of Macht, Green-berg and Isaacs that the intensity of the sound varies as the square of the distance assumes a point source with no reflections from surrounding objects. It is not true for any sort of an en-closed space. For this reason we were interested in the factor of experimental error established for the watch tick tests. It is impossible from the data given to determine the variations in the normal readings because the investigators did not always use the same watch. They were necessarily more interested in ratio diffrences. However one table is offered which indicates the surprising accuracy of the method employed.

The results on single drug effects are summarized in their table 12 on page 163 and which we reproduce as given with one minor correction in the printing, *i.e.,* sodium salicylate should read 74 per cent. instead of 94 per cent.

Acetanilide	78 per cent. normal
Acetphenetidin	129 per cent. normal
Pyramidon	117 per cent. normal
Antipyrine	148 per cent. normal
Lactophenin	139 per cent. normal
Melubrin	150 per cent. normal
Salol	77 per cent. normal
Sodium salicylate	74 per cent. normal
Acetylsalicylic acid	77 per cent. normal
Quinine	110 per cent. normal
Sodium bicarbonate	100 per cent. normal
Aspirin	77 per cent. normal

"It was found that acetanilide, sodium salicylate, acetylsali-cylic acid, phenyl salicylate, and some other drugs decreased the threshold of hearing." "It was found that acetphenetidin, anti-pyrine, pyramidon and some other drugs increased the thresh-old of hearing." The expressions "increased" and "decreased" as applied to the threshold of hearing might in this instance be interpreted in terms of acuity of hearing. The first named group of drugs decreased while the last named group increased the acuity of hearing.

It is extremely important in work on minimum audibility that the variable factor of attention be carefully borne in mind. While no definite information is available on the variable normal for the watch tick tests, the table on the effects of sodium bicar-bonate is extremely interesting. In twelve tests in this table the distances before and after administration are exactly the same.

The range, in the remaining five cases, ran from 6 per cent. minus to 8 per cent. plus. This means that to all intents and purposes seventeen individuals heard the watch at exactly the same distance when the test was repeated in an hour. It would appear, therefore, that Macht, Greenberg and Isaacs had not only overcome the variability in the watch test itself but had also practically eliminated an experimental error in the factor of attention.

The writers determined to check on certain of the antipyretics because it was stated that quinine increased the acuity of hearing. The pitch range of 1000-1800 p.p.s. was used because this particular range was normal for both ears of the subject. Each test was conducted in a similar manner except one (sodium salicylate). The minimum audibility for the scale 1000-1800 p.p.s. was first determnied and then five grains of the drug was taken in a gelatin capsule. The audibility test was repeated in one hour, and at this time a second dose of ten grains was taken. An audibility test was again made after an interval of another hour. We realize that giving the drug in gelatin capsules has certain disadvantages in the matter of absorption but feel this objection is in part overcome by the second double dose.

No appreciable effect was noted in the case of acetanilide, acetphenetidin and pyramidon. By "no appreciable effect" is meant there did not seem to be a difference which lay beyond the amount of experimental error. However, in testing the remaining drugs much smaller steps in energy variations were employed. The results were obtained in absolute values of intensity for audibility under the various conditions. For the sake of comparison, however, the results are given as ratios; the sound intensity required for audition after taking the drug being given in terms of that required before taking. Thus the percentages greater than 100 per cent. denote a decrease in acuity and the figures less than 100 per cent. signify an increase in acuity. Test I was made one hour after the original dose and Test II one hour later as explained above.

Drug	Single Dose		Double Dose	
Salol	Test I.	84 per cent.	Test II.	61 per cent.
Aspirin	Test I.	124 per cent.	Test II.	124 per cent.
Antipyrine	Test I.	77 per cent.	Test II.	77 per cent.
Sodium salicylate	Test I.	(not taken)	Test II.	175 per cent.

These results appear to show that aspirin and sodium salicylate decrease the acuity of hearing, while salol and antipyrine increase the acuity. This is a curious result because according to Macht, Greenberg and Isaacs the action of aspirin, sodium salicylate and salol are practically the same; whereas we seem to find an opposite effect in the case of salol. In the twenty-two cases tested with salol they found only one individual who showed an increase in acuity (1 per cent.) while in a second test this same individual registered no difference whatever. All others read from a minimum of 53 per cent. to a maximum of 91 per cent. We did not feel that our result was due to an idiosyncrasy on the part of our subject and therefore made a comparison of the absolute intensity values involved in the readings of these four drugs.

This gives us a check which is impossible in the cases reported by Macht, Greenberg and Isaacs. It was found that the sound intensity required to hear the scale of 1000-1800 p.p.s. when the acuity had apparently been increased by antipyrine was exactly the same as the amount required when the acuity appeared to be decreased by aspirin. Again the increased efficiency under salol required exactly the same amount of energy as the normal taken before the administration of sodium salicylate. This indicated that variation in normal acuity took place on three successive days. It was decided to find out how much variation might take place in a single day. Accordingly a test for normal was taken at 11 A. M. which we called 100 per cent. intensity. The readings of required intensity for audition taken at 3, 4 and 5 P. M. on the same day without drugs were 88 per cent., 105 per cent. and 135 per cent. respectively. This variation shows a difference of 53 per cent. in two hours and this 53 per cent. covers all of the variations in our figures except that for sodium salicylate.

The results of these experiments are two-fold: first, that these tests, taken under the most favorable experimental conditions, did not yield a difference sufficiently pronounced to be used as a safe basis for deductions on drug effects; and second, all of the variations in amount of intensity lie within a possible error in attention. This same report must be made for morphine sulphate ½ gr.; strychnine sulphate 1-20 gr., and nitroglycerin 1-100th gr. We cannot correlate our findings with the effects as given by Macht, Greenberg and Isaacs.

It was stated in the beginning of this paper that our chief problem was related to the effects of quinine on acuity of hearing. Witmaak (5) has reviewed the literature on this drug and the following notations are from this source:

Roosa in 1873 reported the evidence of an injection of the drum membrane in three colleagues after ten and fifteen grain doses of the drug. Guder in 1880 experimented on twelve normal individuals and denies a hyperemia of the membrane. Witmaak did not find evidence of a congestion in experimental animals and come to the conclusion that quinine has a specific toxic effect on the end organ and cochlear neurones. Ferreri in 1887 reported decrease in both air and bone transmitted sound in a number of cases where large doeses were given (3-4 grams). He found that the acuity of hearing was about as much depressed by 2 grams, as when larger amounts were given. He found also that the hearing returned to normal in about twenty-four hours. It is unnecessary to devote much space to citing instances which confirm the well-known symptoms of quinine intoxication. As has been said we were more concerned in quantitative tests and in particular the relation of the tinnitus to the decreased acuity of hearing. Inadvertently, however, some interesting observations were made in relation to the toxic effects of a large dose of the drug.

The first step in our tests was the establishment of a curve for minimum audibility as a function of pitch. This was obtained in the following manner: Electrical currents of controllable and measureable frequency and intensity were generated by means of a vacuum tube oscillator and amplifier. The harmonic frequencies were eliminated by the use of suitable electrical filters. The thermophone was used as a sound source. This apparatus takes advantage of the heating effect of the current in a thin platinum strip and the consequent expansion of the adjacent air. The sound intensity produced is calculable in terms of the electrical energy input which can be easily measured. The thermophone unit was mounted in a small telephone receiver case which was held tightly to the observers ear under test. A frequency variator of special design was used so that the observer could at will vary the frequency over the range of nearly an octave. This variation

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5 Witmaak, *Arch. Ges Physiol.*, 1903, xcv, 209.

was a continuous one as distinguished from a variation employing discrete frequencies.

The method of procedure in the determination of the minimum audibility was to vary the frequency back and forth over a given range, the intensity being changed by successive increments. For different intensities near the low limit, different portions of the frequency range will in general be audible, and by a suitable choice of intensity increments, the desired curve of relationship between frequency and necessary intensity for audition may be determined as accurately as is desired. Any peaks or dips in the curve which extend over a narrow range of frequencies, will be easily detected by this method. This would not be possible without a prohibitive amount of time and work if determinations were made with a series of separate distinct frequencies, as is usually done. The intensity was first reduced by the operator until the sound was insufficient to be audible to the subject at any point in the frequency range being used. The intensity was then doubled and the subject, by varying the frequency, determined what portion of the range he could hear. This audible frequency range and the corresponding currents being noted, the intensity was again doubled. This process was continued until all the range was heard. A sufficient number of these frequency ranges, each somewhat less than an octave, were used to cover the frequencies from 320 to 3800 p. p. s.

Results are given in terms of "logarithmic sensitivity," this being the logarithm of the reciprocal of the necessary intensity at the limit of audition, the intensity being expressed in absolute units, ergs per square centimeter per second. Thus the high
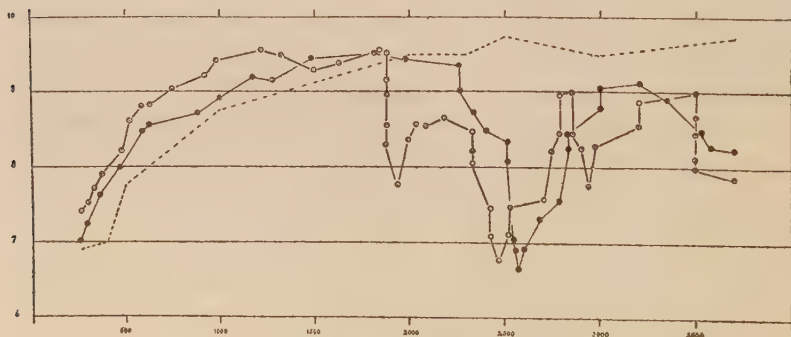

CHART I

parts of the curve represent the greater sensitivities and on the logarithmic scale equal differences in height have much more nearly equal importance in audition than if plotted on a linear scale. Also small values are not obscured at the bottom of the paper. This logarithmic sensitivity seems to be the most logical way to express sensitivity.

Three curves for minimum audibility are presented in Chart 1. The frequencies in the range tested are indicated on the base line and extend from 256 to 3700 p.p.s. The ordinate quantities are expressed in terms of ergs per square centimeter per second and are plotted on a logarithmic scale from 10^{-6th} to 10^{-10th} power. The first curve to be noted is shown by a dotted line and represents the acuity of hearing for the right ear of the subject's fifteen year old daughter taken with the same apparatus. The curve is a normal one and may therefore be used as a guide to the variations from the normal in the two ears of the subject himself. The acuity of the right ear of the subject has been entered as a broken line with light circles while that for the left ear is a continuous line with black circles. The dots on the line indicate the separate frequencies tested and calculated for purposes of the graphic presentation.

The three curves follow a fairly normal picture until the region of 1800 p.p.s. is attained. At no point do the subject's ears differ from that of the control by more than a factor which might be considered beyond a reasonable error in attention. The left ear shows one pronounced spot of decreased acuity (a factor of about 1000 when compared with the control). The lessened acuity begins at 2250 and returns to the normal for this age (43) at 3000. The right ear, on the contrary, shows three areas of decreased acuity; one pronounced spot at 2450 and two less pronounced ones at 1920 and at 2920 p.p.s. These are separated by two peaks, one at 2200 and another at 2800. Both ears display a drop-off at the neighborhood of 3400. (Age change?).

These two curves are interesting because the subject was unaware of any areas of decreased acuity, and even at the present time is not conscious of their presence. The chart gives the reason why the range of 1000-1800 p.p.s. was selected for the tests on antipyretics. If comparisons are to be made of effects on the two ears, this may be done only below the frequency of 1800. The right ear has been chosen for presenting graphically

the effects of quinine. It was thought essential that the drug be studied in reference to the areas of decreased efficiency to find out if they were depressed as much as the more acute regions. Check reading, however, was also made on the left ear with very similar results.

*Test A.*__

Twenty five grains of quinine sulphate was taken in five grain gelatin capsules at hourly intervals. The test began soon after the administration of the last dose. The subjective symptoms at this time were: sensation of fullness in the ears and a slight high-pitched tinnitus. Attempts to locate the period of the tinnitus were unsuccessful. It was our intention to follow the tone scale throughout the range for both ears but this was abandoned because the amount of actual decrease in efficiency did not lie beyond a reasonable error in attention.
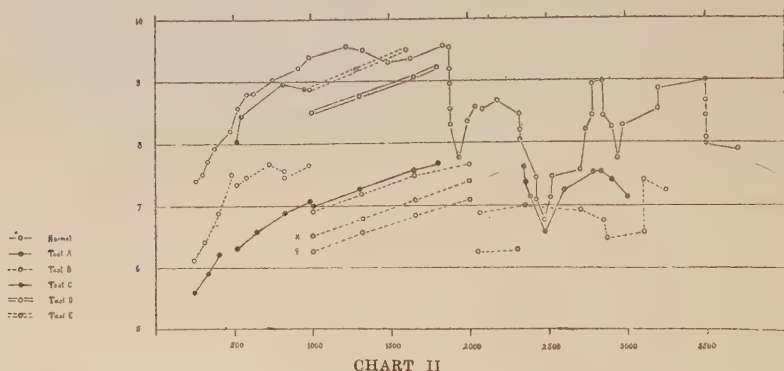
CHART II

The quantities are plotted in Chart II under Test A. While the decreased acuity area at 2460 was located, the peak at 2800 was not identified. Accordingly, it was determined to exhibit a larger dose of the drug.

Test B.

Forty grains of quinine sulphate were taken in 15 grain doses one half hour apart from 9 A.M. to 12:30 P.M. The test began at 3 P.M. Subjective symptons at this time were: a more pronounced tinnitus of the same general character, like the locusts singing in the trees; a more marked sensation of fullness in the ears, and some slight amount of impairment of hearing, particularly for high pitched sounds. This test covered the entire range from 256 to 3250 p.p.s. The results have been entered

on the chart as Test B.   While the line for the range B1 goes over fairly well into that of B2, a sharp drop (factor of about 5) was noted in passing to the range B4, where the departure seemed even more pronounced.   Two subsequent tests were made over the scale B3 yielded the variations as shown in the lines X and Y, (factor of about 10).   Accordingly a final test was made from 2100-2300 (B5) when this same sort of decrease in acuity was again evidenced.

As in the twenty five grain experiment the subject missed the peak at 2800 and also failed to identify the decreased acuity area at 2460.   It was difficult to interpret these variations and three possibilities suggested themselves.   First, the variations might be due to fluctuations in the amount of absorption and excretion of the drug; second, they might be due to errors in attention due to the toxicity of the drug itself in its effect on the nervous system; and third, the pronounced tinnitus might of itself decrease the acuity of hearing.

Test C.

Some four days after Test B. the subject took seventy-five grains of quinine sulphate with the intention of pushing the drug to the limit and also eliminate variations in the amount of absorption by spreading the administration of the drug over a longer period of time.   Thirty-five grains were taken from 6:30 to 9:30 P.M. in seven five grain doses.   Forty grains were taken in the morning in ten grain doses hourly from 6:30 to 9:30. The subject did not find his sleep was materially affected and ate a regular breakfast with enjoyment.   The test was begun at 10:30 A.M. but had to be abandoned for reasons which will be described more completely elsewhere.   The subject became unconscious and was not able to sit up for the test until 4:30 P.M. It was decided to limit the range from 256 - 1800 p.p.s and these readings have been entered on the chart under the letter C. The readings of C1, C2 and C3 line up fairly well.

It appeared from comparing the lower range of Test B with that of Test C that the larger dose influenced the lower part of the range more than it did the upper part, although this is by no means to be accepted as conclusive evidence.

Test D.

This was made on the following morning after a fairly good night's sleep and has been entered under the line marked with

D. This indicated that the subject had practically returned to normal although a pronounced tinnitus was reported.

Test E.

This made at 4:30 the same afternoon is shown which practically coincides with the normal amount of acuity but with the tinnitus about the same as in the morning. The tinnitus gradually disappeared during the next twenty-four hours.

Test F.

There still remained the problem of whether the tinnitus itself masked the note or whether it was the toxic effect of the drug which gave rise to the larger than normal variation in passing from one scale to another. It is well known that tinnitus may be produced by an exposure to loud sounds and it was decided to put this to a test. The subject was placed with his head three feet in front of a telephone receiver which emitted an extremely loud note of 1300 p.p.s. This note was sufficiently loud to be heard outside of the building. After three and one-half hours exposure, the subject was immediately subjected to the test over the range from 1000 - 1800 p.p.s. No material alteration was noted in the acuity over the entire range nor was the exact point of the pitch of 1300 influenced. The tinnitus and sensation of feeling of fullness in the ears was as apparent as in the forty grain quinine test and did not entirely disappear for thirty six hours. This seems to show that while the tinnitus appeared subjectively to distract the attention in the test on quinine, it could not in itself be held responsible for the decreased efficiency of hearing.

Conclusions

We feel the following conclusions may be drawn from these experiments on quinine:

First. A twenty five grain dose did not materially influence the acuity of hearing.

Second. A forty and a seventy five grain dose produced a pronounced decrease in acuity. The larger dose apparently affected the lower part of the scale (256 to 1000) more markedly than the upper part (1000 - 1800). We are, of course, not sure of the amount of drug absorbed and will gladly turn over the subcutaneous or intravenous injection to someone else. There can be no question that the drug was not pushed to the limit in Test C.

Third. The amount of actual loss in acuity of hearing was not proportionate to the loudness of the tinnitus. After hearing returned to normal the tinnitus continued for at least twenty-four hours.

Fourth. A tinnitus produced by a continued loud sound gives rise to similar sensations of fullness in the ears and does not bear any definite pitch relation to the frequency of the sound causing the condition. The acuity of hearing was not impaired after three and one half hours exposure.

Fifth. Recovery of normal hearing after ingestion of quinine occurs in 24 to 36 hours.

Sixth. The toxic affect of quinine influence not only the auditory sense, but the entire nervous apparatus is affected so that definite readings with larger doses are not possible.

Seventh. We present herewith a study in the application of modern methods in the measurement of acuity of hearing. The disadvantages shown in this accurate method practically rule out the watch tick tick and the tuning for K tests in their application to minimum audibility.

70 (2030)

Studies on lung volume. IV. Investigations on admixture of air in the lungs with other air.

By CHRISTEN LUNDSGAARD and KNUD SCHIERBECK.

[*From the Medical Clinic of the University of Copenhagen, Denmark.*]

Several methods of importance for the study of the physiology and pathology of respiration and circulation require (1) that it is possible to produce full admixture of air within the lungs with other air and (2) that the exact conditions necessary for full mixture can be ascertained in a given case. This and the following paper is a short report of experiments dealing with questions.

Technique. 3 liters of oxygen (Allen-Pepys' method) or of hydrogen plus oxygen (Davy-Durig's method) are introduced in

a 5 to 6 liter rubber bag. Starting either from full inspiration or full expiration the subject rebreathes uniformly and almost as deeply as possible from the bag a certain number of times. The connection between the mouth and the bag is a 35 cm. long, 2 cm. wide rubber tube and a 3-way stopcock. The frequency of respiration has always been betwen 8 and 20, usually about 10-15 per minute. During the experiments several small samples (15-20 c. c.) of air were drawn (1) from the bag, and (2) from the very last part of the expiration. In the last instance therefore, alveolar air was obtained. The samples were drawn into evacuated recipients without interrupting or interfering with the breathing. By such a procedure (Fig. 1) one can follow the changes in the composition of the air in the two most important places (bag and alveoli) of the closed system during the rebreathing experiment. In the oxygen experiments the changes in the nitrogen percentage were followed and in the hydrogen experiments the changes in the hydrogen percentage were observed.

Results. In Table I an example of oxygen experiments are given and in Table II an example of hydrogen experiments. Figs. 2 and 3 correspond to Tables I and II respectively.

If complete mixture is defined as a state of uniformity of composition of air in the whole rebreathing system, it is already clear that full mixture cannot be obtained on account of the continuous interchange between the alveolar air and the blood gases, (the influence of respiratory quotient, of hydrogen absorption
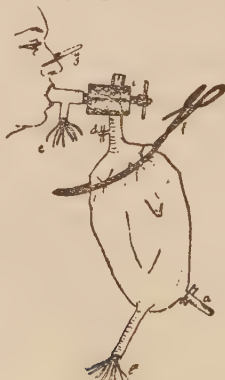
FIG. 1

Five liters rubber bag. Lead tubes with capillary borings for drawing samples without interfering with rebreathing.

and of nitrogen loss by blood). If, on the other hand, complete mixture is defined as the condition where the initial difference between bag air and lung air has disappeared then it is, as our experiments show, possible to obtain such a mixture. An analysis in Table I and Fig. 2 shows that the point of full mix-
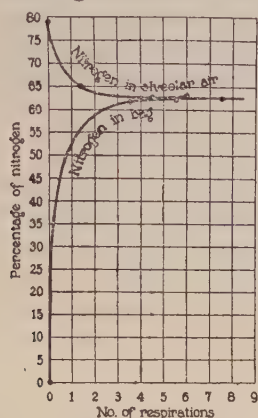
FIG. 2

Mixing curves obtained by taking repeated samples of air from bag (lower curve) and from alveoli (upper curve).

ture is reached when the upper curve is at its lowest point. If rebreathing is continued a steady slight increase in the nitrogen percentage of bag and alveolar air is seen. This is due to the interchange between blood gases and alveolar and can not be overcome by prolonging the experiment. On the contrary, if the rebreathing is continued longer than necessary, the inevitable error due to the alveolar interchange increases. The point of full mixture is therefore indicated by the lowest point of the alveolar curve, and the value for the nitrogen percentage which is most correct is the corresponding point of the lower curve.

------Percentage of hydrogen from last part of expiration (alveolar air)
———Percentage of hydrogen from beginning of expiration
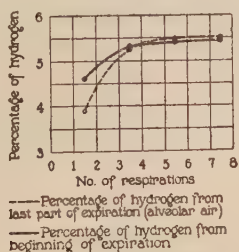
FIG. 3

Mixing curves from hydrogen experiment. Samples taken at the first part (———) and the last part (– – –) of the experiment. Crossing of curves indicates mixture.

In hydrogen experiments similar conditions are obtained if the percentage of oxygen is not too low (table 2, Fig. 3). The two curves cross each other and continue a parallel, slightly upward course. The point which indicates admixture is indicated by the intersection of the two curves. The hydrogen percentage at this point should therefore be used as indicating the percentage in the system at the time of admixture. Our experiments were performed on normal individuals, on emphysematic patients and on one patient with mitral insufficiency. None of the subjects had a vital capacity below 2 liters and a residual air above $2\frac{1}{2}$ liters. In all cases a mixture was obtained between after from 2 to 6 respirations usually after 3 respirations. The error due to the interchanges between alveolar air and blood gases is as a whole not large. It increases with the duration of the experiment and with the degree of difference in tension of the gases in the alveolar air and the blood gases.

TABLE I.

Analyses of samples of air from bag and from alveoli drawn during one rebreathing oxygen experiment.

Number of respiration at which sample is drawn.	Alveolar air.		Air from bag.	
	Nitrogen. per cent.	Carbon-dioxide. per cent.	Nitrogen. per cent.	Carbon-dioxide. per cent.
$1\frac{1}{2}$	64.88	4.39	....	....
$2\frac{1}{2}$	....	....		
$3\frac{1}{2}$			61.72	4.49
$4\frac{1}{2}$	62.46	4.87	....	
$5\frac{1}{2}$	62.61	5.17	62.19	5.10
$6\frac{1}{2}$	....	....	....	....
$7\frac{1}{2}$	....	....	62.72	5.75

TABLE II.

Hydrogen experiment. Analyses of samples of air from the first and the very last part of the expiration. The samples are drawn into evacuated recipients.

Number of respiration at which sample is drawn.	Percentage of hydrogen in the first part of an expiration.	Percentage of hydrogen in the very last part of an expiration.
$1\frac{1}{2}$	4.62	3.90
$3\frac{1}{2}$	5.34	5.30
$5\frac{1}{2}$	5.44	5.48
$7\frac{1}{2}$	5.48	5.52

71 (2031)

Studies on lung volume. V. Quantitative influence of certain factors on admixture.

By CHRISTEN LUNDSGAARD and KNUD SCHIERBECK.

[*From the Medical Clinic of the University of Copenhagen, Denmark.*]

In establishing a complete mixture of air within the lungs with other air several factors come into play. Some of these factors play a minor role and may be varied within rather wide limits before any appreciable influence on the results is observed. Others are of more importance, but their variations can be kept within narrow limits and therefore neglected. There are, however, three factors, which mainly govern the procedure of mixing: first, the number of times rebreathing takes place; second, the depth of the respirations; and third, the amount of air left in the lungs after expiration, that is, the amount of air which can be mixed through diffusion only. The methods where mixture is used, or the subjects on whom these methods are applied, often prevent us from choosing the magnitude of these which would be most suitable for obtaining a complete mixture. In a series of experiments, an example of which are reported in this paper, we have investigated the quantitative influence of variations in these three factors.

Technique. All our experiments are performed on two normal, well trained subjects. We have, for the sake of convenience, exclusively used hydrogen plus oxygen, but earlier experience makes it highly probable that the results can be extended to oxygen, to carbon monoxide, and to nitrous oxide.

A certain varying amount of hydrogen plus oxygen was introduced into a bag or into a Krogh spirometer. The subject started to inspire with a known amount of air left in his lungs. The minimum amount of air left within the lungs was of course the residual air. By starting from different points a known fraction of the reserve air or even of the complimentary air could be added to the residual air. The air left in the lungs was

varied from 1.35 liters to 6.35 liters. Similarly the depth of the respirations, kept constant during an experiment, could be varied regularly from one experiment to another. The whole range was from 1 to 5 liters. The third factor, the number of respirations was varied from 2 to 16. At the end of each experiment the subject expired to the maximum extent and samples were drawn (1) from the bag, and (2) from alveolar air. According to previous investigations a higher hydrogen percentage in the alveoli than in the bag was taken as a proof of full mixture. Table I is an example of one series of 110 experiments. Table II gives all the results in a concentrated form.

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TABLE I.

Example of experiments. Complete mixture is present if $b =$ or $> a$.

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Experiment number.	Amount of air left in lungs after expiration.	Depth of respirations.	Number of respirations.	Hydrogen in sample from spirometer (or bag). a	Hydrogen in sample from alveolar air. b	Difference between two samples. $b - a$
	liters	liters		per cent.	per cent.	
1	4.85	1.5	9	11.97	11.90	—0.07
2	4.85	1.5	10	6.32	6.36	+0.04
3	4.85	2.0	6	7.50	7.43	—0.07
4	4.85	2.0	7	9.28	9.29	+0.01
5	4.85	3.0	4	9.54	9.48	—0.06
6	4.85	3.0	5	11.06	11.00	—0.06
7	4.85	3.0	6	10.82	10.82	±0.00
8	3.85	1.5	5	15.25	14.90	—0.35
9	3.85	1.5	6	11.51	11.51	±0.00
10	3.85	1.5	7	16.01	16.03	+0.02
11	3.85	2.0	5	9.12	8.91	0.21
12	3.85	2.0	6	13.15	13.17	+0.02
13	3.85	3.0	4	10.15	10.05	—0.10
14	3.85	3.0	5	10.60	10.60	±0.00
15	3.85	4.0	2	10.47	10.13	—0.34
16	3.85	4.0	3	12.94	12.91	—0.03
17	3.85	4.0	4	10.29	10.30	+0.01
18	3.85	4.0	5	16.52	16.52	±0.00

TABLE II.

Relations between amount of air left in lungs after expiration, depth of
respiration and number of respirations in experiment giving full mixture
of lung air with hydrogen in two normal individuals.

Amount of air left inside lungs after expiration (residual air plus more or less of vital capacity). *liter*	Depth of respirations. *liter*	Number of respirations necessary for securing full mixture.
(a) Rebreathing from a Krogh spirometer.		
6.35	1.5	14
5.85	1.5	13
	2.0	7
4.85	1.5	10
	2.0	7
	3.0	6
3.85	1.5	6
	2.0	6
	3.0	5
	4.0	4
2.85	1.5	6
	2.0	5
	3.0	5
	4.0	4
	5.0	3
2.00	1.0	9
	1.5	6
	2.0	4
	3.0	3
	4.0	3
	5.0	3
(b) Rebreathing from five-liter rubber bag.		
2.00	1.0	6
	1.5	5
	2.0	3
	2.5	3
	3.0	3
1.30	1.0	7
	1.5	5
	2.0	3
	2.5	3
	3.0	3

72 (2032)

Studies on lung volume. VI. The absolute and relative size of the different lung volumes.

By CHRISTEN LUNDSGAARD and KNUD SCHIERBECK.

[*From the Medical Clinic of the University of Copenhagen, Denmark.*]

In 27 normal adults, 19 men, 8 women, age 19 to 36 years, total capacity, vital capacity, residual, complementary and reserve air were determined. Vital capacity was determined by expiration into a Krogh spirometer. The highest value of five determinations was chosen. Reserve and complementary air and middle capacity were determined by rebreathing air plus oxygen from a Krogh spirometer. Total capacity was determined by the dilution method (rebreathing oxygen). That complete mixture took place was proved by construction of mixture curves. Residual air was determined directly by the dilution method and indirectly as the difference between the total capacity and the vital capacity. The highest and lowest values are recorded in Table I. The values in this and the following 3 papers are given at observed tension and room temperature.

If the total lung volume is given a value of 100, we obtain an average value for the other volumes as shown in Table II, where we have included the corresponding values calculated from previous investigator's publications. We have determined the mean error of each determination by means of the usual formula

$$f = \sqrt{\frac{\Sigma_i \zeta^2}{(n-1)}}.$$ The values for the mean, for the mean error and for the mean error in percentage of the mean are given in Table III.

In order to find out whether or not any systematic error was responsible for the deviations from the mean, we compared the observed and calculated number of errors in the different groups. The agreement was satisfactory. In Table IV are given the relative size of the lung volumes. If we know the normal value of one of the lung volumes, the normal values for the other may be calculated by means of the figures in Table IV. We do not

put much stress on the middle capacity, the reserve and the complementary air, on account of the uncertainty in the determination of these figures.

The other figures are more important, and the relation of 4 — 3 — 1 of the total capacity, vital capacity, and residual air may prove of value in future work.

TABLE I

Highest and lowest absolute values for lung volumes in 27 normal adults arranged according to total capacity.

		Total capacity.	Middle capacity.	Residual air.	Vital capacity.
		liter	liter	liter	liter
18 men	maximum	7.82	4.89	1.97	5.85
	minimum	4.84	3.09	1.34	3.50
9 women	maximum	4.83	2.91	1.06	3.77
	minimum	3.08	1.88	0.88[1]	2.20

[1] In two other women the residual air was 0.75 liter and 1.38 liter.

TABLE II.

Relative values of different lung volumes.

Authors.	Material	Total capacity. T	Middle capacity. $\frac{M}{T} \times 100$	Residual air. $\frac{R}{T} \times 100$	Vital capacity. $\frac{V}{T} \times 100$
Bohr (1906)	9 men 1 woman	100	58.2	23.2	76.8
Rubow (1908)	8 women	100	56.0	26.3	73.7
Lundsgaard and Van Slyke (1918)	10 men 5 women	100	58.8	24.8	75.2
Present paper	19 men 8 women	100	62.0	24.7	75.3

TABLE III

Mean error and mean error in per cent. of mean value of relative lung volumes.

	Number of determinations.	Mean value.	Mean error on each determination.	Mean error in per cent. of mean value.
$\frac{M}{T} \times 100$	25	62.0	3.5	5.6
$\frac{R}{T} \times 100$	27	24.7	4.0	16.2

TABLE IV.

Normal relative values for the different lung volumes based on 27 observations.

Total capacity....................	100	T
Middle capacity................	62	$T \times \dfrac{62}{100}$
Residual air......................	24.7	$T \times \dfrac{24.7}{100}$
Vital capacity..................	75.3	$T \times \dfrac{75.3}{100}$
Reserve air........................	37.3	$T \times \dfrac{37.3}{100}$
Complementary air..........	38.01	$T \times \dfrac{38.0}{100}$

73 (2033)

Studies on lung volume. VII. Relation of size of chest to lung volume.

By CHRISTEN LUNDSGAARD and KNUD SCHIERBECK.

[*From the Medical Clinic of the University of Copenhagen, Denmark.*]

In 1918 Lundsgaard and Van Slyke[1] worked out the quantitative relationship between the different lung volumes and the size of the chest (so-called chest volume) in 18 normal individuals. The size of the chest was determined as the product ("chest volume") of three dimensions, the height, depth, and width of the thorax. The ratio between the chest volume and the lung volume in the corresponding position was found to be 55 for maximum expiration, 37 for middle capacity, and 19 for maximum inspiration. We thought it would be of value to increase the number of observations. We used the technique described by Lundsgaard and Van Slyke in measuring the chest dimensions and the total capacity. Complete mixture has always

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[1] Lundsgaard, C., and Van Slyke, D. D., *J. Exp. Med.*, 1918, xxvii, 65.

been present. The residual air we determined indirectly as the difference between total capacity and vital capacity and the middle capacity was determined by rebreathing from a spirometer.[2] The material includes 27 normal subjects (18 men and 9 women). The figures for the relative lung volumes reported in a previous communication, are based on the same observations. We obtained the following average figure (Table I). It is in approximate agreement with the figures obtained by Lundsgaard and Van Slyke (Table II). The discrepancies are probably due to the fact that the middle capacity, the vital capacity, and the residual air are determined in a slightly different way. (See Lundsgaard and Van Slyke, and Lundsgaard and Schierbeck.[3] We believe that the procedure adopted in this paper is the more preferable. The mean error on each determination helps in deciding whether or not an observation in a patient may be considered pathological or not. The ratio between the "chest volume" in after maximum inspiration and maximum expiration respectively indicates the range of the thorax movement.

TABLE I.

Average values for ratios of "chest volumes" to lung volumes in 27 normal subjects. Ratio of "chest volume" after maximum expiration to "chest volume" after maximum inspiration. Mean error and mean error in percentage of average figure.

Position of chest and lungs.	Abbreviation.	Number of subjects.	Average figure.	Mean error.	Mean error in percentage of average figure
$100 \times \dfrac{\text{Total lung volume.}}{\text{"Chest volume" after full inspiration.}}$	$\dfrac{V_t}{C_t} \times 100$	27	55.7	3.1	5.6
$100 \times \dfrac{\text{Middle lung capacity.}}{\text{"Chest volume" in same position.}}$	$\dfrac{V_m}{C_m} \times 100$	25	40.3	4.4	10.9
$100 \times \dfrac{\text{Residual air.}}{\text{"Chest volume" after full expiration}}$	$\dfrac{V_r}{C_r} \times 100$	27	18.3	3.2	17.5
$100 \times \dfrac{\text{Vital capacity.}}{\text{"Chest volume" in resting position.}}$	$\dfrac{V_v}{C_m} \times 100$	27	49.1	6.7	11.6
$100 \times \dfrac{\text{"Chest volume" after full expiration}}{\text{"Chest volume" after full inspiration}}$	$\dfrac{C_r}{C_t} \times 100$	27	74.7	5.0	6.7

[2] Lundsgaard, C., and Schierbeck, K., Paper No. 6 of this series.
[3] Lundsgaard, C., and Schierbeck, Paper No. 6 of this series.

TABLE II.

Ratio between "chest volumes" and lung volumes. For explanation see Table I.

Author.	Number of observations.	$\dfrac{V_t}{C_t} \times 100$	$\dfrac{V_m}{C_m} \times 100$	$\dfrac{V_r}{C_r} \times 100$	$\dfrac{V_v}{C_m} \times 100$
Van Slyke and Lundsgaard	18	54.1	37.9	18.6	45.
Present paper	27	55.7	40.3	18.3	49.1

74 (2034)

Studies on lung volume. VIII. Patients with heart disease (mitral lesions).

By CHRISTEN LUNDSGAARD and KNUD SCHIERBECK.

[*From the Medical Clinic of the University of Copenhagen, Denmark.*]

The lung volumes were determined in 11 adult patients with mitral lesions. Three of the patients were in the uncompensated state of the disease. Nine were in the compensated stage. In these last patients the second pulmonary sound was markedly accentuated indicating an increased pressure in the pulmonary circulation. Our technique was as previously described.[1] Care was taken to secure full mixture in determining the total capacity. All the lung volumes are given at room temperature and observed pressure as in previous publications of the series.

Results. Discussion will appear more complete elsewhere [2, 3] and only the main results will be given here. Relative lung volumes are given in Table I in percentage of the normal relative value for total capacity, middle capacity, residual air, and vital capacity, respectively (100, 62.0, 24.7, and 75.3) established in a previous paper.[4] These values show in all instances

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[1] Lundsgaard, C., and Schierbeck, K., Paper No. 6 of this series.

[2] Lundsgaard, C., *Journ. Amer. Med. Ass.*, 1923.

[3] Lundsgaard, C., and Schierbeck, K., Paper No. 6 of this series.

a decrease in the vital capacity and an increase in the residual air. The values for the middle capacity vary. These results are in accordance with the results of other investigators.

Calculated normal values and observed values. The normal values for the different lung volumes in each patient are calculated from the size of the chest which was determined according to the procedure of Van Slyke and Lundsgaard. On account of the diminished range of chest movement only the ratio $(\frac{54}{100})$ for the total lung volume was used. The other lung volumes were calculated by means of the normal ratios for the relative lung volumes (100 — 62 — 24.7 and 75.3) previously published. This mode of calculation was adopted on account of the diminished range of chest movement and because we have every reason to believe that the maximum inspiratory expansion is normal in these cases. However, the lung volumes based on all the normal ratios did usually not differ materially from those reported in Table II. In Table II the directly observed lung volumes are given in percentage of the calculated normal figures. The information gained in this way gives quite another picture than the (so to speak distorted) one we get by using the relative values. The total capacity is either normal (mild cases) or decreased (more advanced cases). The middle capacity follows as a whole the total capacity. The residual air is decreased in patients in the decompensated stage, but increased in the compensated. The vital capacity is in all instances decreased but through a different mechanism in the two types. This observation makes us understand the mechanism of the lung involvment in stasis. In the mild cases an emphysematic condition takes place, probably on account of stiffness of the pulmonary vessels through increased blood pressure in the lungs. (Cf. v. Basch's experiments.) In the advanced cases some of the space for the residual air is taken up by overloading of the lungs with blood and edematous fluid, the residual air is therefore diminished. Figure I gives our conception of the condition. A is the calculated normal volume for Patient No. 1. B and C give the observed volume. The conditions found are, in our opinion, brought about not by the mechanism shown in B, but as shown in C, where the black area indicates the space in the chest taken up by (1) increased size of heart, (2) increase in amount of blood

in lungs, (3) increase in amount of lung tissue, including possible intraalveolar and intrapleural exudate and (4) increased size of abdominal organs, causing diminished downward movement of diaphragm.

TABLE I.

Observed lung volumes given as relative values based on total capacity.

Number of patients.	Total capacity.	Middle capacity.	Residual air.	Vital capacity.	Remarks.
1	100	105	122	92	Patients with incompensated heart failure.
2	100	99	143	85	
3	100	90	118	94	
4	100	9.5	145	85	
5	100	87	146	84	
6	100	142	156	81	
7	100	....	215	62	Patients with compensated heart failure.
8	100	103	170	77	
9	100	101	136	88	
10	100	95	135	88	
11	100	125	187	71	

Normal ratio for thorax excursion 74.7. See paper V of this series.

TABLE II.

Observed values of different lung volumes in per cent. of normal value. Calculated from the size of the chest wall.

Number of patients.	Total capacity.	Middle capacity.	Residual air.	Vital capacity.	Excursion of thorax. $\dfrac{C_r}{C_t}$	Remarks.
	per cent.	per cent.	per cent.	per cent.		
1	60	63	73	56	86.4	Patients with incompensated heart failure.
2	52	51	73	45	84.7	
3	54	49	64	52	82.5	
4	78	78	112	67	83.0	
5	100	87	145	86	79.2	
6	71	100	109	58	80.3	Patients with compensated heart failure.
7	81	......	172	50.5	82.0	
8	85	87	142.5	65	81.5	
9	102	103	132	94	80.9	
10	107	102	142	96	84.3	
11	89	112	164	61	85.3	

Normal ratio for thorax excursion 74.7. See paper V of this series.
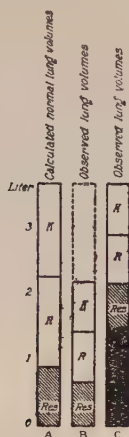
1. Diagram showing mode of production of changes in total lung volumes in heart patients. From Patient No. 1. Res. = residual air. R = reserve air. K = complementary air.

75 (2035)

Studies on lung volume.   IX.   Patients with lung emphysema pulmonum.

By CHRISTEN LUNDSGAARD and KNUD SCHIERBECK.

[*From the Medical Clinic of the University of Copenhagen, Denmark.*]

Twelve patients suffering from chronic emphysema of the lungs were studied. All of them except Nos. 5 and 11 have also asthma but no determinations were made within 24 hours after an attack of asthma. Except in No. 5 no fine rales were present in the lungs. The technique was as described in previous papers. In the diluting experiments, full mixture was secured by constructing "mixtures curves" (see paper No. 4 of this series). All figures in this and previous papers are given at room temperature and observed pressure. The size of chest was determined as described by Lundsgaard and Van Slyke. The normal total lung volume was calculated as $\dfrac{54}{100}$ × observed "chest volume" in maximum

inspiratory position. The other lung volumes were calculated by means of the normal ratio of the different lung volumes (see paper VI). In the table the observed lung volumes are given in percentage of the calculated normal figures. The movement of the diaphragm was observed by X-ray. In all but two, Nos. 4 and 6, a diminished excursion of chest wall (procedure described in paper VII was found. In most instances a diminished excursion of the diaphragm was found (by fluoroscopy). Fig. 1, representing the conditions in Patient No. 1, typifies the conditions found in the majority of patients with emphysema pulmonum.

TABLE I.

Observed lung volumes in 12 patients with emphysema given in percentage of calculated normal lung volumes.

Number of patients	Total capacity.	Middle capacity.	Residual air	Vital capacity.	Excursion of thorax expressed as $\dfrac{C_r}{C_t} - 1$
	per cent.	per cent.	per cent.	per cent.	
1	101	121	200	69	78.3
2	105	114	117	101	75.2
3	99.	126	124	91	81.4
4	98	86	126	88	74.7
5	89	105	176	60	88.5
6	99	103	79	106	74.0
7	96	99	137	80	81.4
8	99	103	153	82	86.6
9	104	95	104	106	77.4
10	101	103	125	95	75.5
11	104	123	149	88	81.8
12	99	......	161	78	80.4

[1] Normal ratio 74.7. (See paper V).
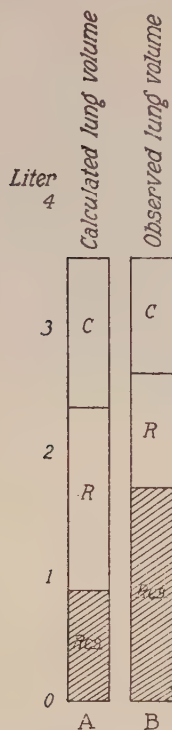
1. Diagram showing calculated and observed lung volumes in patient No. 1 may typify the changes usually found in uncomplicated emphysema of the lungs. Res = residual air. R = reserve air. C = complementary air.

76 (2036)

Cholesterol determination in duodenal contents.

By J. J. HERTZ and MAX KAHN.

[*From the Department of Laboratories, Beth Israel Hospital, New York City.*]

The duodenal contents of fasting patients were examined before and after the administration of a saturated solution of magnesium sulfate, according to the method of Lyons. The Duodenal tube was allowed to remain in the duodenum for a

period of two hours and the contents examined every ten minutes. It was found that usually there is a distinct increase in the cholesterol present in the duodenal contents after the administration of the magnesium sulfate. After an hour the cholesterol content decreases.

The amount of cholesterol in the duodenal contents varies in different patients, between 25 and 105 milligrams per 10 c. c. in the fasting state. After the administration of the magnesium sulfate, the amount of cholesterol is often tremendously increased. This increase is very sudden and would give the impression as if the gall bladder voided its concentrated bile into the duodenum.

77 (2037)

The organic constituents of the saliva.

By HOWARD B. LEWIS and HELEN UPDEGRAFF.

[*From the Laboratory of Physiological Chemistry of the University of Michigan, Ann Arbor, Mich.*]

We have applied the recent method of Benedict for the determination of uric acid in blood to the filtrate obtained from saliva by a slight modification of the Folin-Wu tungistic acid precipitation method. Fifty-one samples of saliva from thirty healthy men showed maximum and minimum contents of uric acid of 2.9 and 0.6 mgs. per 100 c.c. of saliva, while fifteen samples from ten women showed similar variations from 2.3 mg. to 0.7 mg. In more than fifty per cent. of the salivas obtained from men, the uric acid content fell within a range of 1.6 to 2.1 mgs., while in the case of the women the values ranged from 1.05 to 1.15 mgs. in more than fifty per cent. of the salivas examined. Twenty-two samples collected at intervals over a period of four months from the same woman showed variations of from 1.5 mg. to 0.7 mg. Samples of saliva and blood were collected simultaneously and analyzed for uric acid.

Salivary uric acid was much lower than the uric acid of the blood (approximately thirty per cent. of the uric acid of the blood in most cases) as shown in the table.

The salivary glands are apparently not readily permeable to the glucose of the blood. In only one of the salivas studied was there obtained sufficient reduction (Folin-Wu method) to make possible an approximate estimation (0.006 per cent. calculated as glucose). Although the blood sugar was increased about fifty per cent. one and one-half hours after enteral administration of 100 grams of glucose, glucose could not be detected in the salivas of two individuals.

Further work on other organic constituents of saliva is in progress.

COMPARATIVE URIC ACID CONTENTS OF SALIVA AND BLOOD.

Subject	Sex	Uric acid per 100 c.c.	
		Saliva.	Blood.
		mg.	mg.
E. H.	F.	2.2	3.9
H. U.	F.	1.2	4.0
H. U.	F.	1.1	3.9
H. U.	F.	1.5	4.1
H. B. L.	M.	1.3	5.3
H. B. L.	M.	2.2	5.6
H. B. L.	M.	2.1	7.1
H. B. L.	M.	2.1	7.5
W. H. G.	M.	1.7	3.2
G. H. H.	M.	1.8	4.1
M. B. T.	M.	1.3	4.4

<div align="center">

78 (2038)

A note on the antiseptic properties of olive oil.

By JUSTINA H. HILL (by invitation) and DAVID I. MACHT.

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[*From the Brady Urological Institute and the Pharmacological Laboratory, Johns Hopkins University.*]

While studying the antiseptic properties of various benzyl compounds described elsewhere,[1] the authors examined a number of drugs which were insoluble in water, in order to test their bactericidal or antiseptic properties. Solutions of these drugs were made in various oils and the effects of the oily solutions were studied on staphylococcus pyogenes aureus. In this connection some interesting observations on the effect of the oils themselves were noted which were deemed worth while reporting in this place. In order to determine the antiseptic efficiency of the drugs in oil check or control experiments were made with the oils themselves by the following method.

Twenty-four hour broth cultures of staphylococcus pyogenes aureus were used for these tests, the cultures being filtered through glass wool before use to remove clumps of organism. 2 c.c. of the sterile oil to be tested was inoculated with one standard loopful of culture. Specimens for plating were removed at the end of 1 minute, 1 hour, 3 hours and 5 hours, each specimen being 0.1 c.c. drawn from the emulsion by means of a capillary pipette attached by a rubber stopper to a Tuberculin syringe. Dilutions in two parallel series were made in sterile 0.875 per cent. sodium chloride solution. Agar plates were made from these dilutions, and after 48 hours at 37.5 C°., the two parallel plates of the same dilution which showed the most suitable number of colonies for counting were selected, the colonies counted and the number of bacteria per c.c. estimated.

The following oils were examined; olive oil, cottonseed oil, liquid petrolatum or albolene (mineral oil), peach kernel oil and oil of sweet almonds. The results obtained are expressed in the subjoined table. It will be noted that olive oil exhibited an action very different from all the other oils studied. It was distinctly antiseptic and germicidal. The number of organisms was rapidly decreased after one hour and the cultures were com-

pletely sterilized at the end of five hours.   Albolene after an initial drop at the end of one hour, showed a marked increase in the number of organisms after that time.   Cottonseed oil and peach kernel oil showed an increase of organisms at the end of the first hour.   Oil of almonds although greatly reducing the number of organisms in five hours showed a marked increase in organisms before that time.   The most striking observation in this study is the antiseptic effect of olive oil.   This could not be attributed to impurity or acidity of the oil as only the purest product, neutral in reaction, was employed in the tests.   The above findings may be of interest to such clinicians as larynologists, rhinologists and others who have occasion to use solutions of antiseptics and other drugs in oil.

ACTION OF OILS AGAINST STAPHYLOCOCCUS PYOGENES AUREUS

Oil.	1 minute.		1 hour.		3 hours.		5 hours.	
	Number of organisms per c.c.	Per cent.	Number of organisms per c.c.	Per cent.	Number of organisms per c.c.	Per cent.	Number of organisms per c.c.	Per cent.
Olive oil	110,000	100	16,500	15.	1,350	1.2	0	0.
Cotton seed oil....	136,000	100	145,000	106.6	30,910	22.7	25,450	18.7
Albolene	59,000	100	1,500	25.4	67,500	144.4	67,500	144.4
Oil of almonds....	1,400,000	100	5,150,000	367.8	250,000	17.8	16,000	1.1
Peach kernel oil..	530,000	100	1,160,000	218.8	1,885,000	355.6	440,000	83.0

79 (2039)

On the estimation of organic phosphorus.

By EMIL J. BAUMANN.

[From the Laboratory Division, Montefiore Hospital, New York City.]

In attempting to use the methods of Bloor and Bell and Doisy for estimating liopoid phosphorus, difficulty was experienced in securing uniform results.   A study of these methods was therefore made using pure inorganic phosphate solutions.   Complete recovery was rarely possible and the apparent losses were quite variable.   After excluding other steps in the processes as possible

causes of error, attention was given to the ashing process, modifications of the Neumann ashing process being used. In this method, which had given satisfaction for the macro estimation of phosphorus for a long time, two possible sources of error were discovered.

Although when heated alone, phosphoric acid does not volatilize until a temperature of about 260° C. is reached, when it is heated with sulphuric acid at 180°-200° C. it volatilizes slowly in the vapors of sulphuric acid—a fact which the writer subsequently found had been demonstrated by Hillebrand and Lundell. This source of loss was found to occur both in the Bloor and Bell and Doisy methods. In addition, when the latter method of ignition was used, some conversion of ortho to pyrophosphoric acid was usually found to occur because of the very small amount of sulphuric acid used.

The fact that volatilization occurs under the conditions present in the Bell and Doisy and Bloor ashing processes was demonstrated as follows:

One c.c. of a phosphate solution containing 1.0 mg. of P was placed in the bottom of a pyrex test tube 8 x 1 and to it were added a few washed quartz pebbles, 8 drops of sulphuric acid and 1 c.c. of nitric acid as suggested by Bell and Doisy, or 1.5 c.c. of a mixture of equal parts of sulphuric and nitric acids as Bloor uses. The upper part of the tube was then drawn out and bent at right angles and the tubes heated as in the ashing processes of these authors. The vapors were collected in a dish of water. The solution containing the vapors was concentrated on the water bath and the acid neutralized with freshly distilled ammonium hydroxide. The amount of phosphorous was then estimated in the distillate and residue. Losses of from 2 to 15 per cent. were found to occur. A typical experiment with the Bloor ignition method is given:

Amount of phosphate used	1.00 mg.
" " " in distillate	.07
" " " " residue	.94

When the Bell and Doisy ignition method was used the sum of the phosphorus in the distillate and residue was usually less than the amount started with due to conversion of ortho to pyrophosphoric acid.

To avoid these sources of error, several other oxidizing agents were tried in the hope that a lower temperature for oxidation might be used. Redistilled 30 per cent. hydrogen peroxide was finally chosen. (I am indebted to Dr. I. Greenwald for bringing this reagent to my attention). The material is oxidized in a large test tube covered with a watch glass, with 8 drops of sulphuric acid and 0.2 c.c. of the peroxide. Additional hydrogen peroxide is added if necessary—two or three drops at a time.

From this point various methods may be used for the estimation. We have proceeded as follows: The contents of the tube are washed into an evaporating dish and concentrated on a water bath until most of the water is removed. The solution is then transferred to a graduate and the phosphorus estimated according to the colorimetric process of Bell and Doisy.

For estimating lipoid phosphorus of blood, the Bloor extraction has been used while other tissues have been dried with plaster of Paris and extracted with ether and alcohol.

No difficulty has ever been experienced in securing complete recovery from pure phosphate solutions by the proposed process. A comparison of results obtained by this method and by that of Bell and Doisy with inorganic phosphate solutions and tissue extracts follows:

	Proposed method.	Bell and Doisy.
1 mg. sodium phosphate	1.01 mg.	0.93 mg.
0.2 mg. sodium phosphate	0.20 mg.	0.16 mg.
Tissue Extract A	0.31 mg.	0.30 mg.
B	0.36 mg.	0.34 mg.
C	0.28 mg.	0.24 mg.
D	0.15 mg.	0.14 mg.
E	3.02 mg.	2.62 mg.
F	1.50 mg.	1.35 mg.

It should be added that precautions must be observed in redistilling hydrogen peroxide; the distillation is done under diminished pressure and the distillate protected from coming in contact with rough surfaces or organic matter.

All glassware must be very carefully washed with distilled water and all reagents except the carbonate-sulphite solution must be tested to be certain that they are phosphate free.

80 (2040)

The effects of graded saturation of the circulatory blood on the respiratory response to the administration of carbon dioxide and on the total oxygen consumption of the dog.

By ROBERT GESELL, CHARLES S. CAPP, and FREDERICK FOOTE.

[*From the Department of Physiology of the University of California, Berkeley, California.*]

Saturation of the blood with carbon monoxide resulted in an increased pulmonary ventilation both on the administration of room air and of a mixture of carbon dioxide in room air. Desaturation led to a return to normal respiratory response, though not always complete.

The effects of saturation of the blood with carbon monoxide on the rate of oxygen consumption were variable. In some instances the rate of oxygen consumption decreased progressively from the outset of saturation. In other instances this decrease in the rate of oxygen consumption was preceded by a temporary increase in the rate of oxygen consumption.

This observation along with the finding that the respiratory response to the administration of carbon dioxide was not as constant as that noted with hemorrhages[1] points to circulatory compensations which are absent if the blood volume is less than normal.

Since the effects of carbon monoxide poisoning and hemorrhage are in general the same they are apparently due, in both instances, to similar disturbances in oxidation and transport of blood gases.

The results obtained on saturation of the blood with Carbon monoxide, therefore, support our view[2] on the significance of the coordination of the dual function of hemoglobin and the volume flow of blood in relation to the mechanism of the chemical control of respiration.

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1 Gesell, Capp and Foote, PROC. SOC. EXP. BIOL. AND MED., 1921, xix, 1; *The American Journal of Physiology*, 1922, lxiii, 1.

2 Gesell, Foote and Capp, *The American Journal of Physiology*, 1922, lxiii, 32.

81 (2041)

Action of drugs upon the central nervous system of insects.

By G. F. PILZ and W. J. CROZIER.

[*From the Zoological Laboratory, Rutgers College, New Brunswick, N. J.*]

Peculiarities revealed by study of the action of neurophil drugs in lepidopterous larvæ[1,2] led to an extension of the observations to include an imaginal insect. Effects following injection of solutions of drugs into the thorax of grasshoppers were compared with those attending the direct application of these substances to the thoracic ganglia (exposed by removal of the ventral thoracic sclerites). In all essentials, the two methods gave comparable results.

As in caterpillars, strychnine produces momentary general excitation, but only when present in very high concentration; it fails to induce "reversal of inhibition." In the grasshopper, however, such "reversal" appears after administration of nicotine or of camphor. The legs are thrown outward and upward if (under nicotine) the abdomen or a femur be touched, and (under camphor) when the mouth-parts are stimulated tactically. The grasshopper normally clasps a stimulating object if, as in these tests, the animal be held by a clip fastened upon the wing-covers. Nicotine, and pilocarpine, stimulate especially movements of the two posterior pairs of legs, while under camphor all three pairs and the mouthparts undergo spasmodic movements.

Grasshoppers, caterpillars, and crayfish[3] agree in presenting definite evidence of neuronic excitation by these substances: strychnine, pilocarpine, picrotoxin, nicotine, veratrine, atropine, caffeine, camphor, phenol. To this general correspondence there may be added certain aspects of detailed agreement. These arthropods differ pronouncedly from the earthworm in the fact that, with the latter,[4] nicotine, caffeine and phenol fail to pro-

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1 Crozier, W. J., PROC. SOC. EXP. BIOL. AND MED., 1922, xix, 326.

2 Crozier, W. J., *Biol. Bull.*, 1922, xliii, 239.

3 Moore, A. R., PROC. SOC. EXP. BIOL. AND MED., 1922, xix, 335.

4 Moore, A. R., *Jour. Gen. Physiol.*, 1921, iv, 29.

duce central nervous stimulation.   There is reasonable ground, therefore, for the opinion that the central nervous systems of arthropods possess certain common features, revealed through the actions of neurophie drugs; and that these features distinguish the arthropod central nervous system from that of an oligochaete.

82 (2042)

Tallowiness in butterfat.

By GEORGE E. HOLM and G. R. GREENBANK.

[*From the Research Laboratories of the Dairy Division, Bureau of Animal Industry, Department of Agriculture, Washington, D. C.*]

Butterfat exposed to light and air rapidly takes on an odor and off flavor which has been termed "tallowy."   Among the early workers who attributed the tallowness in fats to the direct action of oxygen are Winckel,[1] Scala,[2] Ryan and Marshall,[3] Vintilesco and Popesco,[4] and others.   Winckel attributes such a state to the action of oxygen upon oleic acid, but he was not able to show the reactions in butter and cocoanut oil that he showed in other fats.   Vintilesco and Popesco were apparently the first to postulate the direct union of oxygen with the unsaturated linkages of fats to form peroxides which readily release their oxygen in the presence of peroxidases, giving reactions with guaiacol.

Smith[5] favors the view that rancidity is induced by enzymes, while Hunziker and Hosman[6] attribute tallowness in butter to oxidation with subsequent splitting and the formation of fatty acids and glycollic acid.   Palmer and Combs,[7] more recently, favor the view that tallowness in butter is dependent

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[1] Winckel, M., *Apothekers Ztg.*, 1905, lxix, 690.

[2] Scala, A., *Staz. spermi. agric. Ital.*, 1897, xxx, 613.

[3] Ryan, L. A., and Marshall, J., *Am. Jour. of Pharm.*, 1907, lxxix, 308.

[4] Ventilesco, J., and Popesco, A., *J. de Pharm. et d. Chimie*, 1915, xii, 318.

upon the natural oxidases present, while Rogers[8] concludes from his studies that the changes in butter "is brot about by spontaneous chemical action."

The latter workers mentioned have dealt mainly with factors influencing the rate of formation of tallowness in butter.

The problem of the oxidation of butter was studied from another standpoint by Dyer,[9] who measured the rate of disappearance of oxygen in stored butter.

Numerous products of oxidation of butter have been isolated and identified, but the nature of the oxidation which occurs has never been satisfactorily explained.

Results of our work show that the reactions of tallowy butterfat substantiate the view that peroxides are formed. Such fats have the power of liberating iodine slowly from potassium iodide, and the amount liberated in a certain length of time, as shown by the $Na_2S_2O_3$ titre, forms a comparative test of how much oxygen has been absorbed by various fats.

These fats also give the Kreis test, seemingly in direct proportion to the amount of oxygen which they have absorbed. Extreme cases of oxidation have been found, however, in which little or no iodine is liberated from potassium iodide, but the Kreis test is very pronounced. Oxidized butterfats also give a peroxide test with chromic acid when acidified and allowed to stand for a comparatively long time.

It has been found that oleic acid exposed to oxygen for some time will give the characteristic tallowy odor and proportionate peroxide and Kreis tests. Triolein under similar conditions will give an even more characteristic odor and both the named reactions. Its tendency toward oxidation seems less than that of oleic acid.

Unsaturated linkages other than those found in the oleic radical may be involved in the oxidation, but the oleic acid radical is undoutedly one of the constituents of butterfat largely involved in the production of tallowiness.

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5 Smith, H. L., *Pharm. Jour.*, 1915, xcv, 4.

6 Hunziker, O. F., and Hosman, D. F., *Jour. Dairy Sci.*, 1917, i, 321.

7 Palmer, L. S., and Combs, W. B., *Jour. Dairy Sci.*, 1919, ii, 444.

8 Rogers, L. A., *Third Internat. Congress of Refrigeration*, Washington-Chicago, 1913.

9 Dyer, D. C., *J. Agr. Research*, 1916, iv, 927.

83 (2043)

The presence and determination of adenine nucleotide in human blood.

By HENRY JACKSON, JR.

[*From the Department of Medicine of the College of Physicians and Surgeons, Columbia University, and the Presbyterian Hospital, New York.*]

Tungstic acid protein free blood filtrate was hydrolyzed with dilute mineral acid for two hours, then made strongly alkaline with ammonia and evaporated slowly to a small volume. After filtration, the filtrate was made alkaline with ammonia and precipitated with silver chloride in ammonia. The washed precipitate was decomposed and picric acid added. The resulting crystaline precipitate was purified and one analysis proved to have 29.3 per cent. nitrogen. It melted at 281 C. After removal of the picric acid, the residue proved to have approximately 38 per cent. nitrogen. It precipitated with gold chloride and ammoniacal silver. It gave no color with Nessler's solution and no blue color with alkaline phosphotungstate.

Tungstic acid filtrate was precipitated with silver, the precipitate broken up and the filtrate precipitated with neutral lead acetate in acid solution. The lead precipitate was decomposed and then gave a strong pentrose reaction with orcin and an absorption band between the D and the E. With napthoresorcin the color produced did not shake out with ether or benzol. There was no xanthin test nor was there any trace of blue with phosphotungstate.

If the blood filtrate was first hydrolyzed with acid and then the same procedures carried out no traces of pentrose reaction could be found.

If the blood filtrate was precipitated with silver, then lead, and the filtrat from lead sulphide was hydrolyzed with acid subsequent addition of silver precipitated the nitrogen containing portion of the substance, while the substance producing the pentrose reaction remained in the filtrate.

If the inorganic phosphates are removed from the filtrate from lead sulphide there is no immediate test for phosphates

with ammonium molybdate. If, however, this same filtrate, free from inorganic phosphates be hydrolyzed with dilute acid before the test is done a yellow precipitate characteristic of phosphates appears immediately.

To tungstic acid blood filtrate was added under definite conditions uranium acetate. The washed precipitate was decomposed and the resulting filtrate on digestion gave a nitrogen content of about 4 mgm. of nitrogen per 100 c.c. whole blood. Blanks were negative. Adenine nucleotide added to blood was recovered quantitatively by this method.

Conclusions. Adenine, probably bound is present in considerable quantities in normal human blood. There is some evidence presented to show that it is bound in the form of adenine nucleotide.

A method is outlined whereby nucleotides may be determined quantitatively in small samples of tungstic acid blood filtrate.

It is suggested that a large part of the undetermined nitrogen in the tungstic acid filtrate of Folin and Wu is adenine nucleotide.

The work is being continued at The Thorndike Memorial Laboratory of the Boston City Hospital.

ABSTRACTS OF COMMUNICATIONS, MINNESOTA BRANCH

Eighth meeting.

Minneapolis, Minnesota, December 13, 1922

84 (2044)

A statistical study of the form and growth of a diphtheroid bacillus.

By ARTHUR T. HENRICI.

[*From the Department of Bacteriology and Immunology, University of Minnesota, Minneapolis, Minn.*]

In a previous communication[1] I described changes in the size of the cells of *Bacillus megatherium* during the growth of a

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1 PROC. SOC. EXP. BIOL. AND MED., 1921, xix, 132.

culture, and variability in size, particularly the bimodality of the frequency curves in the early stages of growth. Further observations of this organism, varying the number and age of the cells in the inoculum showed that these changes are constant but vary in degree, the increase in size, and variation in size, and the tendency to bimodality being greater with smaller and older seedings; and that, while these changes take place during the vegative phase of the culture, there is apparently no actual correlation between the variations in size and the rate of cell division. It is noteworty that the coefficient of variation increases and decreases with the size of the cells.

I have made a similar study of a chromogenic diptheroid bacillus isolated from lake water. It is larger than most members of this group of bacteria, but like the rest of the group decreases in size during the vegetative stages of growth and increases during the resting period; the curve for size is therefore just the reverse of that for *B. megatherium*. The decrease in size began after a latent period and during the logarithmic growth phase. As the cells decreased in size the frequency curves became more symmetrical and the variation decreased; as the size increased again the curves became more extended and showed skewness. There was no tendency to bimodality. the single mode gradually shifting. The coefficient of variation decreased with the actual decrease in size, *i.e.*, during the period of most rapid cell divisions. Therefore the coefficient of variation cannot be used as an index of rate of reproduction with organisms such as these, where the entire population is subject to fluctuations in size independent of the growth of the individuals from youth to maturity. The numbers of metachromatic granules also decrease during the period of active growth and increase during the resting period, and there is a positive correlation between the size of the cells and the numbers of granules. But the number of granules decrease more rapidly and extensively than the size of the cells, and therefore the coefficient of correlation is high during the resting phase and low during the vegetative phase.

85 (2045)

Organ weights in albino rats with experimental rickets.

By C. M. JACKSON and RACHEL CARLETON.

[*From the Department of Anatomy, University of Minnesota, Minneapolis, Minn.*]

Of the 118 rats used for this work, 37 were normal controls and 81 were young rats which had been fed various diets by Professor McClendon to produce experimental rickets.[1] The 81 test rats were autopsied and classified on the basis of gross skeletal appearance and previous X-ray examination as follows: 27 apparently normal, or nearly so; 19 with slight rickets; 19 with moderate rickets; and 16 with severe rickets. The diagnosis was also confirmed by microscopic examination, although the histological changes were found somewhat variable.

The weights of the various organs and parts were compared with the norms for corresponding body length or weight established by Hatai, Jackson and Donaldson. The percentage deviation was calculated for each organ and averaged for the five groups. The chief results are briefly summarized.

The body weight and tail length appear nearly normal (for corresponding body length) in all. In the rachitic rats the organs may be grouped as follows:

A *decrease* occurs in the weight of the integument, hypophysis, dry skeleton, empty stomach and intestine, and especially in the thymus.

An *increase* occurs in the weight of the eyeballs, heart, gastrointestinal contents, and especially in the submaxillary glands, kidneys and suprarenals.

No regular changes of importance were noted in the weight of the head, ligamentous and cartilaginous (moist) skeleton, musculature, brain, lungs, liver, spleen, ovaries, testes and epididymides, although marked variations occur in some groups.

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1 PROC. SOC. EXP. BIOL. AND MED., 1922, xix, 356.

86 (2046)

The dominant reacting tissues in anaphylactic, peptone and histamine shock.

By W. H. MANWARING, R. C. CHILCOTE, W S. CLARK and R. E. MONACO.

[*From the Laboratory of Experimental Pathology, Stanford University, California.*]

Canine anaphylactic shock, peptone shock and histamine shock are currently assumed to be physiologically identical reactions. In each shock there is a sudden, pronounced fall in arterial blood pressure, the carotid pressure being reduced to about 25 mm. Hg. by the end of two minutes. Recovery usually begins about the tenth minute, the arterial pressure being restored to normal in from 30 minutes to 90 minutes, depending upon the severity of the reaction. In each shock, fatal results may be produced by the injection of large doses or by the use of highly sensitized animals. In each shock there is a pronounced splanchnic engorgement and cyanosis, the production of hemorrhagic lesions in the intestinal mucosa, and a reduction in blood coagulability. In order to test the assumed physiological identity of the three shocks, we have endeavored to determine the topographical distribution of the dominant reacting tissues in each shock.

1. *Canine anaphylactic shock.* Anaphylactic shock (fall in arterial blood pressure) does not take place in dehepatized (Eck-fistula) dogs. This is not only true for the mildly sensitized dogs previously reported,[1] but is equally true for highly sensitized dogs giving the fatal type of the reaction. Practically no change in arterial blood pressure is produced in these highly sensitized animals, even on the intravenous injection of as large a dose as 30 c.c. of specific foreign (horse) serum. The liver

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[1] Manwaring, W. H., Der physiologische Mechanismus des anaphylaktischen Shocks, *Zeitschr. f. Immunitätsf.*, 1910, viii, 1.

is therefore not only the dominant but the essential reacting organ in canine anaphylactic shock.

2. *Canine peptone shock.* The severity of the peptone reaction is reduced in dehepatized dogs, and is further reduced in completely eviscerated dogs. There are therefore important though not dominant hepatic and intestinal factors in this shock. In dehepatized dogs, recovery from the shock does not take place, the animals dying in about 60 minutes. The liver, therefore is the essential or dominant organ in peptone recovery.

3. *Canine histamine shock.* The severity of the histamine reaction is not reduced in dehepatized dogs nor in eviscerated dogs. The dominant reacting tissues in this shock, therefore, are either confined to the extra-hepatic and extra-intestinal parts, or are fairly evenly distributed throughout the body as a whole. Recovery from histamine shock takes place as promptly and completely in dehepatized and eviscerated dogs as in intact animals.

Canine anaphylactic, peptone and histamine shock, therefore, are not physiologically identical reactions, at least in so far as their initial or fundamental physiologic mechanisms are concerned. The secondary reactions due to low systematic blood pressure are presumably identical in the three shocks. In the later stages of each shock, the secondary reactions conceivably dominate the clinical picture.

87 (2047)

Histamine reactions in isolated canine tissues.

By W. H. MANWARING, R. E. MONACO, and H. D. MARINO.

[*From the Laboratory of Experimental Pathology, Stanford University, California.*]

Marked histamine reactions may be demonstrated by perfusion methods in isolated canine organs. The following are the reactions thus far studied:

1. *Isolated hind-quarters.* Distinct decrease in perfusion resistance (vaso-dilation), increasing the rate of perfusion flow from 15 per cent. to 200 per cent, depending upon the initial

vascular tone of the parts. At the height of the reaction, the perfusion rate is identical with the perfusion rate with amyl nitrite. Marked edema of the hind quarters, especially of the genitalia.

2. *Isolated intestines.* Distinct increase in perfusion resistance (vaso-constriction), decreasing the perfusion rate from 15 per cent. to 50 per cent., depending upon the histamine concentration used. Marked peristaltic movements during the first three minutes of the test, followed by edema, peritoneal transudation, and increased volume of intestinal contents.

3. *Isolated liver.* Marked increase in perfusion resistance (vaso-constriction), decreasing the perfusion rate as much as 90 per cent. with large histamine doses (1:25000). Edema, peritoneal transudation.

4. *Isolated lungs.* Marked increase in perfusion resistance (vaso-constriction), decreasing the perfusion rate from 50 per cent. to 75 per cent. depending upon the histamine concentration used. Marked pulmonary edema.

Histological study of these reactions will be reported later.

88 (2048)

The hepatic mechanical factor in canine anaphylactic shock.

By W. H. MANWARING, R. C. CHILCOTE, and SELLING BRILL.

[*From the Laboratory of Experimental Pathology, Stanford University, California.*]

It has been assumed by certain observers[1] that the sudden pronounced fall in arterial blood pressure, the characteristic feature of acute anaphylactic shock in dogs, is due to a reduction in the available systemic blood volume as a result of splanchnic engorgement. This engorgement they believe is a passie congestion due to hepatic obstruction. We have endeav-

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1 Weil, R., and Eggleston, C., *Jour. Immunol.*, 1916, ii, 525. Simons, J. P., *J. A. M. A.*, 1919, lxxiii, 1437.

ored to test this theory by studying the effects on carotid blood pressure of a mechanical obstruction to hepatic outflow sufficient to produce a combined hepatic and intestinal passive congestion equal to the passive congestion observed during anaphylactic shock. We have taken the increase in portal blood pressure as the measure of this passive congestion.

The normal portal blood pressure averages about 9 mm.Hg. in our series of dogs. This pressure is increased to about 18 mm.Hg. during anaphylactic shock, the maximum being reached by the end of one minute. The portal blood pressure then gradually falls, and is restored to normal in from 8 minutes to 15 minutes.

To prepare animals for the mechanical test, the inferior vena cava was ligated immediately below the liver in a series of dogs. Examination of these dogs six weeks later, at the time of the tests showed an hypertrophied collateral circulation fully compensating for the vena caval ligation.

To make the tests, an unclosed ligature was placed about the vena cava immediately below the diaphragm. By partially closing this ligature, any desired degree of hepatic-intestinal passive congestion could be produced without interfering with the return circulation from the hind quarters. It was found that carefully controlled increased resistance to hepatic outflow, sufficient to raise the portal blood pressure to 20 mm.Hg., which is greater than the maximum portal pressure during anaphylactic shock, was without marked effect on the carotid blood pressure. The carotid pressure usually falls slightly on tightening the ligature, but is restored practically to normal by the end of two minutes. It is only when the vena caval ligature is completely closed so as to produce combined hepatic and intestinal stasis, that a fall in carotid pressure is produced at all comparable with the fall during anaphylactic shock.

We conclude from these tests, that hepatic-intestinal passive congestion, though conceivably a factor of some importance in canine anaphylactic shock, is not the essential or dominant factor in this shock. This finding strengthens our initial theory[2], that the anaphylactic reaction in dogs is essentially an explosive hepatic autointoxication, the formation or liberation of hepatic products having a histamine-like reaction on the extra-hepatic blood vessels.

[2] Manwaring, W. H., *Zeitschr. f. Immunitätsf.*, 1910, viii, 1; *J. A. M. A.*, 1921, lxxii, 849.

ABSTRACTS OF COMMUNICATIONS

Fourth meeting.

Western New York Branch, Clifton Springs N. Y.,
December 16, 1922.

89 (2049)

Vital capacity determinations in persons with normal heart and lungs above forty years of age.

By D. C. WILSON,   (by invitation).

[*From the Clifton Springs Sanitarium, Clifton Springs, N. Y.*]

The first vital capacity readings were taken by Hutchinson in 1846 on 2,000 persons of all ages by means of a spirometer. His subjects were not given a physical examination. He found the vital capacity to vary with the height and weight. He also stated that the vital capacity increased 1 cu. in. yearly up to the age of 35 and after 35 it decreased 1 cu. in. yearly. Since his time the body surface has been found to measure more closely the vital capacity variations. However, all normal persons studied have been babies, students or young adults. No normal readings have been above forty when the surface area is used as the standard for variation.

The present study is an attempt by accurate physical examination, fluoroscopy of the chest and blood studies to secure patients of all ages who have no cardiac or lung disease. Next to obtain by three separate readings their true vital capacity. Then to compare this by means of a Du Bois chart with the so-called normal for their body surface area. Eighty-five such cases are reported and the results given.

Except for women overweight and between 45 and 55 years of age, the surface area reading is within 500 c.c. of the reading obtained unless there is some cardiac or lung disease. This rule does not hold above the age of 70 when there is great individual variation. There is no such regular decrease in vital capacity after 35 as Hutchinson mentions.

90 (2050)

Urine acidity after the injection of adrenalin chloride.

By ROGER S. HUBBARD.

[*From the Clifton Springs Sanitarium, Clifton Springs, N. Y.*]

The following experiment was planned to study the effect of adrenalin chloride upon the acidity of the urine, and to determine, if possible, whether the "acidosis" which has been described as one of the results of such injections was due to the respiratory changes which follow the administration of the drug. A normal man (the author) who weighed 165 pounds took a standard simple breakfast on each of four successive days. On each day hourly samples of urine were collected throughout the morning. The collection and determination of the degree of acidity was carried out by the method of Marshall[1]; other determinations were done as described in previous papers[2]. On the second day of the experiment 1 c.c. of a 1 to 1000 solution of adrenalin chloride (Parke-Davis) was injected subcutaneously into the arm. Samples of blood were collected from the median basilic vein one half hour before and one half, one, and two hours after the injection was given.

TABLE I

TIME	NOTES	URINE					BLOOD		BREATH
A.M.		Vol. c.c	Sp. Gr.	Reac. P_H	NH_4N mg.	T. N. mg.	CO_2 vol. per cent.	Sugar per cent.	CO_2 mm.
7:00	began								
8:00		35	1.030	5.6	8.7	666			
8:40	breakfast								
9:00		25	....	5.8	5.0	447			
10:00		36	1.032	7.3	10.0	575	63.3	0.111	36
10:35	adrenalin								
11:00		56	1.031	7.4	9.8	659	51.9	0.172	29
11:35							51.9	0.270	
12:00		37	1.022	6.2	11.4	449			32
12:35							51.9	0.111	
1:00		34	1.027	5.6	10.8	554			

[1] *J. Biol. Chem.*, 1922, li, 3.

[2] Hubbard and Wright, *J. Biol. Chem.*, 1921, xlix, 385; Hubbard and Nicholson, *ibid.*, 1922, liii, 209.

The results are given in the tables. Table 1 shows that the subject developed the decrease in the carbon dioxide combining capacity of the plasma described by Peters and Geyelin[3], and Hubbard and Wright (loc. cit.) as well as a rise in blood sugar. The determinations of the alveolar carbon dioxide tension were not very satisfactory, but they showed a decrease which roughly paralleled the changes in the carbon dioxide combining capacity of the plasma.

TABLE II

TIME.	VOLUME.		SP. GR.		REACTION.		AMMONIA N.		TOTAL N.	
	cont. c.c.	exp. c.c.	cont.	exp.	cont. P_H	exp. P_H	cont. mg.	exp. mg.	cont. mg.	exp. mg.
7–8	34 31 32.5	35	1.037 1.032 1.020	1.031	5.7 5.4 5.6	5.6	5.6 9.6 33.0	8.7	524 469 974	666
8–9	34 31 31.5	25	1.034 1.032 1.018	....	6.7 5.35 5.6	5.8	4.8 8.7 11.5	5.0	486 577	447
9–10	28.5 22 59	36	1.031 ...... 1.020	1.032	7.35 5.6 6.5	7.3	3.6 7.3 19.0	3.6	418 705	575
10–11	29 40 48	56	1.029 1.030 1.024	1.031	7.1 6.0 6.9	7.4	4.8 10.0 10.4	9.8	387 728 475	659
11–12	42 32 54	37	1.029 1.027 1.025	1.022	6.8 5.55 6.6	6.2	6.2 9.1 16.6	11.4	529 279 567	449
12–1	.... 33 50	34	.... 1.023 1.025	1.027	.... 5.4 6.2	5.6	...... 7.9 14.3	10.8	...... 540 574	554

Under "cont." (control) are listed the results from the experiments when no adrenalin was taken. These results are listed in sequence.

Under "exp." (experiment) are listed the results obtained on the second day—the day on which the adrenalin was given.

Table 2 shows the determinations made on the urine on the day when adrenalin was given contrasted with similar determinations on the three control days. The acidity of the urine alone showed any change, and that change was slight. The reaction of the specimen collected from eleven to twelve o'clock after the adrenalin was given showed a more marked increase in acidity (1.2 P_H) over the specimen collected during the preceeding hour than did similar specimens on the control days.

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3 J. Biol. Chem., 1917, xxxi, 471.

This comparatively rapid passing of the "alkaline tide" was the only change in urine reaction found. The experiment certainly did not show an increased alkalinity of the urine after the administration of adrenalin comparable to that which follows polypnea[4], but further experiments with increased amounts of adrenalin would be necessary to prove definitely that an increased urinary acidity results from the use of the drug. The marked tremor and other disagreeable symptoms experienced during the experiment make the undertaking of such investigations upon human subjects a rather serious matter.

The experiment reported furnishes some evidence that the lowered carbon dioxide combining capacity of the plasma which follows injection of adrenalin chloride does not resemble, in some of its accompanying phenomena, that which is produced by polypnea.

91 (2051)

Ingested fat and body fat as precursors of the acetone bodies.

By ROGER S. HUBBARD.

[*From the Clifton Springs Sanitarium, Clifton Springs, N. Y.*]

An experiment was carried out to determine whether body fat and ingested fat give rise to equal amounts of the acetone bodies. A normal woman took a diet which furnished twenty per cent. more calories than her calculated basal requirement, and which consisted of 45 grams of protein, 45 grams of carbohydrate, and 160 grams of fat. The excretion of aceto-acetic acid, β-hydroxybutyic acid, and nitrogen were determined daily by methods previously described[1]. The amounts of the acetone bodies excreted were ten to twenty times the amounts excreted by the subject when she was on a normal diet, but the total amounts (0.25 grams of acetone from all of the acetone bodies)

4 Collip and Backus, *Am. J. Physiol.*, 1920, li, 568; Grant and Goldman, *ibid.*, 1920, lii, 209.

1 Hubbard and Wright, *J. Biol. Chem.*, 1922, l, 361.

were small.   The diet used should not have caused any excretion
of acetone if Shaffer's formula[2] is applied; the combustion of
varying mixtures of ketogenic and antiketogenic compounds in
different parts of the organism and at different times during
the day will probably give rise to such amounts of acetone as
these when the total amount of antiketogenic material is some-
what in excess of the ketogenic.

After six days on the diet described the fat was discontinued
for three days, and the same amounts of protein and carbohy-
drate were fed as before.   During this time body fat was pre-
sumably burned to replace the fat omitted from the diet.   The
diet fed at the beginning of the experiment was then resumed
for a period of two days.   There were no differences in the
amounts of acetoaecetic acid, β-hydroxybutyric acid, and nitrogen
excreted in the three periods.   Samples of blood taken before
breakfast before the experiment began, after six days on the
high fat diet, and after three days on the low fat diet contained
the same amounts of cholesterol (determined by the method of
Bloor[3] and of fat (determined by the methods of Bloor[4] and
of Gage[5].

From these results it was concluded that body fat and in-
gested fat (at least when the latter is not present in very great
amounts) gives rise to the same amounts of the acetone bodies
when metabolism takes place under comparable conditions.

[2] *J. Biol. Chem.*, 1922, liv, 399.

[3] *J. Biol. Chem.*, 1916, xxiv, 227.

[4] *J. Biol. Chem.*, 1917, xxxi, 375.

[5] *Cornell Veterinarian*, 1920, x, 154.

92 (2052)

Observation on the origin of biotypes (microbic dissociation) in pure lines of bacteria.

By RALPH R. MELLON.

[From the Highland Hospital, Rochester, N. Y.]

The experiments reported show that bacterial variability can be identified with the pleomorphic cycle.   The strain studied was a bacillus that formed giant coccoids under certain conditions.   The morphologic and staining changes occurring in these are indicative of biochemic reorganization that conditions variability in the strain, However, variability only occurs if the coccoids are transplanted to an environment that is selective enough to further the changes that are indicated by the pleomorphism itself.

In this case the pleomorphic giant coccoids when transplanted at 20 C° grew after about two weeks, when usually they grow best at 37° in 24 to 48 hours.   Under the former conditions a bacillary variant was produced instead of the original bacillus.   The variant was a distinct type morphologically and culturally, although immunologically it agglutinated the antiserum for the original bacillus better than the homologous strain itself did.   The titre of the latter was 640, for the variant 1,280.   The variant also absorbed all the agglutinins from the serum.

This strain has been under observation for six years as a pure line culture, and is prone to form rudimentary branching forms as well as giant coccoids.   The reorganization occurring in the giant coccoids made possible the dissociation of the branching phase as a distinct type.   Pleomorphism for this strain is regarded as a true life cycle, and in reality represents potential variation.   The biologic nature of the giant coccoids is shown in the following study.

93 (2053)

Observations on the relation of bacterial giant coccoids to zygospore formation.

By RALPH R. MELLON.

[*From the Highland Hospital, Rochester, N. Y.*]

The giant coccoids of the strain in the above paper have previously been shown to have been the precursors of a diplococcus mutant[1]. Their viability is much greater also than that of the bacillary form. Such important functional differences lead to an inquiry regarding the nature of these structures. *B. coli* and its congeners, as well as diphtheroids, have been studied. Employing an environment that led the strains to reproduce in their fungoid or branching phase, a mechanism was demonstrated that was practically identical morphologically with zygospore formation as it occurs among the yeasts.

A very striking concomitant change was a spiral reorganization of the chromatic substance which took place in the filamentous forms which formed the zygospore. This was often so striking as to resemble the strands of a rope, such structures probably being identical with the so-called giant whips described first by Novy many years ago. Similar formations among the protozoa are spoken of as the "wurstformige Schlingen" which represents the most constant accompaniment of the sexual process among the protozoa.

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[1] *Jour. Med. Res.*, 1920, xlii.

94 (2054)

The blood pressures and heart rate, in girls, during adolescence. Biometrical constants for 1,700 cases.

By STANLEY ROSS BURLAGE (by invitation).

[From the Department of Physiology, Cornell Medical College, Ithaca, N. Y.]

In a previous paper before this Society[1] the writer discussed the source and methods used in obtaining this data.

The biometrical constants determined were, the correlation coefficients, means, standard deviations, each with its probable error, and the probable error of a single observation.

Correlation of systolic pressure, diastolic pressure, pulse pressure and pulse rate was made with age in years, height in inches and weight in pounds. These constants were determined for six different groups of data, *i. e.*, schoolgirls who had not reached puberty, schoolgirls who had reached puberty, total schoolgirls, schoolgirls who had reached puberty and college girls, college girls, and finally the total schoolgirl and college girl population.

The numerical values of the constants will be published elsewhere and only the general conclusions given here.

Conclusions:

1—Blood pressure and pulse rate data for schoolgirls and college girls must be correlated separately. That for schoolgirls must further be separated into a group of girls who have reached puberty and a group who have not.

2—When systolic pressure is correlated with weight, height and age:

    a—Schoolgirls who have not menstruated show the greatest correlation with the factors, weight, height and age, in the order named.

    b—Schoolgirls who have reached puberty show correlation, in this order, with weight and height but none with age. These coefficients are less than those in (a).

    c—College girls show significant correlation only with weight.

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1 Burlage, S. R., PROC. SOC. EXP. BIOL. AND MED., 1922, xix, 247.

3—When diastolic pressure is correlated with weight, height and age:

  a—Schoolgirls who have not reached puberty show the greatest correlation with the factors, weight, height and age, in the order named. The correlation however is not as great as that found with systolic pressure.

  b—Schoolgirls who have reached puberty show correlation in the same order as in (a) but the coefficients are less in each case.

  c—Here too, the college girls show correlation with weight only, and this coefficient is less than that for systolic pressure.

4—When pulse pressure is correlated with weight, height and age:

  a—The schoolgirls who have not reached puberty show correlation with the factors in the order named. These coefficients are less than those for diastolic pressure.

  b—Neither the schoolgirls who have reached puberty nor the college girls show significant correlation with any of the factors.

5—When pulse rate is correlated with height, age and weight:

  a—The schoolgirls who have not reached puberty show correlation with the factors in the order named.

  b—The schoolgirls who have reached puberty show a correlation with age and weight, in this order.

  c—The college girls show no significant correlation with any of the factors.

  Of course these correlations with pulse are negative correlations.

6—When considering the data from girls who have reached the age of puberty, there seems to be in most cases, a better correlation, in all of the series studied, in the schoolgirl group than in the college girl group.

95 (2055)

A case of hyperglycemia in a thyroidectomized sheep.

By A. BODANSKY, SUTHERLAND SIMPSON, and S. GOLDBERG.

[*From the Laboratory of Physiology and Biochemistry, Cornell University Medical College, Ithaca, N. Y.*]

A series of experiments was undertaken recently to determine the effects of administration of thyroxin, thyroid extract, and iodine upon thyroidectomized and normal sheep. A study of the blood sugar was to form a part of the research.

Preliminary examinations yielded the following typical data: Normal sheep, 60-68 mgms. per 100 cc.; thyroidectomized sheep, 51-57 mgms. per 100 c.c. The data for each sheep varied within a narrower range.

One thyroidectomized sheep (No. 11), the subject of this report, showed a condition of hyperglycemia, instead of the typical hypoglycemia. While the figures varied within a wider range, they were all definitely above the normal level. Three successive analyses yielded the following values: 78, 70, and 84 mgms. per 100 c.c.

The effect of thyroxin upon No. 11 has been compared with that upon normal and typical thyroidectomized sheep. The effect upon normal sheep (1W) was immediate and gradual, rising to a maximum of 84 mgms. per 100 c.c. on the 10th day. One of the thyroidectomized sheep (No. 1) showed an immediate rise from its basal figure of 55 mg. to 76 mg. per 100 c.c., continuing on to a maximum of 83 on the 5th day. Another (No. 4) rose more gradually, and with fluctuations, to 71 mgms. per 100 c.c. on the 14th day.

No. 11, the experimental cretin showing hyperglycemia, went from his average of 77 mg. per 100 c.c. to 86 mg., showing 87 mg. 3 days later and then dropping to a very much lower range (53-71 mg. per 100 c.c.), again behaving atypically.

A definite explanation of the hyperglycemia and the subsequent drop of blood sugar had to be deferred until a post mortem. Hypertrophy of the adrenals or degeneration of the isles of Langerhans were among the possibilities to be investigated.

Comparative data showed hypertrophy of the adrenals. No. 11's glands weighed 4.91 gms. (right) and 5.22 gms. (left) as compared with 1.5-2 gms. in a normal sheep.

Histological examination showed hyperemia and hemorrhage in the adrenals, which may explain the drop. The pancreas was normal.

Pathological examination showed the immediate cause of death apparently pulmonary oedema. All the fat of the body showed myxoedema which did not give any fat reaction with Herxheimer's stain. The entire extent of the aorta and the pulmonary arteries, together with the larger branches, showed calcified plaques in the media next to the intima. The intima was normal. Sections of these did not give any fat or iron reactions. In the heart there was myxoedema of the subepicardial fat and scattered areas where the myocardium had apparently reverted to an embryonic type with large nuclei and a large amount of cytoplasm with longitudinal striations of the periphery. There was also marked hydropericardium and hydrothorax. The kidneys showed congestion of the glomeruli and an enormous amount of colloid in the Bowman capsules, in the convoluted and collecting tubules. The adrenals showed marked hyperemia and hemorrhage areas. There was a considerable amount of cortical tissue out of proportion to the medullary tissue. (Hyperemia and hemorrhage of the adrenals have been ascribed to toxemia). The pancreas seemed to contain a normal number of islands of Langerhans.

96 (2056)

Effects of thyroxin, thyroid extract, and sodium iodide, respectively, on neuro-muscular activity in cretin sheep.

By HOWARD S. LIDDELL and SUTHERLAND SIMPSON.

[*From the Department of Physiology and Biochemistry, Cornell University Medical College, Ithaca, N. Y.*]

Thyroxin was administered to four cretin sheep in the form of daily subcutaneous injections and its influence on the spontaneous activity of the animals was estimated by means of a pedometer attached to the fore leg. One cretin had been thyroidectomized at about six weeks of age and showed extreme muscular weakness four months later. Daily subcutaneous injections of one half milligram of thyroxin was followed after an interval of nine or ten days by a marked increase in activity. One year later, injections of 0.25 mg. of thyroxin every second day were again followed in three days by a pronounced increase in neuro-muscular activity. Another cretin sheep of about the same age as the first and thyroidectomized at the same time received daily injections of one fourth milligram of thyroxin during a period of prostration four and one-half months after the extirpation of the thyroid. In this case the rise in the activity curve occurred three days following the first injection. One year later, thyroxin was again administered in doses of 0.25 milligrams every second day and the sudden increase in activity began after a latent period of six days.

The other two cretin sheep (one of which had previously thyroxin, the other having been fed thyroid extract) both gave essentially the same reaction as the two preceding sheep to daily injections of one-half milligram of thyroxin. The latent periods were in one case, five days for the first series of injections and four days for a similar series of thyroxin injections eleven months later. In the other case, the interval between the beginning of treatment and a marked increase in activity was six days.

One cretin sheep less active than the normal, showed no increase in activity following the ingestion of one-half gram of sodium iodide every second day for a period of sixty-three days. In the case of another thyroidectomized sheep which was fed

0.3 gram of thyroid extract every second day, the activity was maintained above that of the normal sheep but no sudden and pronounced increase was observed.

97 (2057)

Influence on the respiratory metabolism of pancreatic extract administered by mouth to depancreatized dogs.

By C. B. F. GIBBS and JOHN R. MURLIN.

[*From the Physiological Laboratory of the University of Rochester, Rochester, N. Y.*]

Extracts of beef pancreas prepared in the same manner[1] as extracts of dog's pancreas were prepared by Murlin and Kramer[2] in 1913-6 and administered by mouth to depancreatized dogs with glucose and NaOH in sufficient quantity to make the entire mixture N/20 NaOH produced in all of four trials distinct elevations of the respiratory quotients taken from two to four hours afterward. In two of the four trials the extract was concentrated and purified before administration and in both instances the rise in quotient was greater than when crude unconcentrated extract was given.

One of the experiments on a depancreatized dog is reproduced below.

Dog No. 40.   Operated Nov. 28th, 1922.

Date.	Time.	CO_2	O_2	*RQ*
Dec. 1	12:40-1:35	2.943	4.271	0.689
	1:35-2:47	4.073	6.151	0.662
	2:47-3:48	3.330	4.799	0.694
Dec. 2	11:05-11:43	1.960	2.596	0.755
	11:43-12:13	1.578	1.959	0.721
	12:13-12:46	1.619	2.508	
	3:40 P.M.	Dog given 50 c.c conc. No. 87(2) extract in 300 c.c water + 20 gm. glucose + N/20 NaOH (final reaction).		
	4:53-5:23	1.497	2.854	0.757
	5:23-6:23	2.932	3.813	0.769
	6:23-7:41	3.966	4.479	0.885
	7:41-8:42	3.284	3.424	0.959

[1] Murlin, Kramer and Sweet, *Journ. Metabolic Research*, 1922, ii, 19.
[2] Murlin and Kramer, *Journ. of Biol. Chem.*, 1916, xxvii, 516.

SCIENTIFIC PROCEEDINGS

98 (2058)

Some relations between hydrogen-ion concentration and antigenic properties of proteins.[1]

By I. S. FALK and M. F. CAULFIELD.

[*From the Department of Public Health, Yale School of Medicine,
New Haven, Conn.*]

The recent work of J. Loeb[2] and others has indicated the important rôle which the hydrogen-ion concentration plays in affecting the physical and chemical properties of proteins. Our studies were designed to determine whether certain biological properties of the proteins were also affected by hydrogen and hydroxyl-ions over the range of concentration which markedly affects such properties as osmotic pressure, swelling power, viscosity, power to combine with ions, electrical charge, etc.

Our first studies upon the anaphylactogenic properties of proteins were made with gelatin. We failed to produce anaphylaxis in guinea pigs with this protein when it was introduced intravenously or intraperitoneally in solutions at its isoelectric point

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[1] These studies were aided by a grant from the Loomis Research Fund of the Yale School of Medicine.

[2] Loeb, J., Proteins and the Theory of Colloidal Behavior, New York, 292 pp.

(P_H = 4.7) and in more acid and in more alkaline solutions. With pure, crystallized hen ovalbumin (isoelectric point, P_H = 4.8) we obtained anaphylaxis in the guinea pig readily and consistently. When this protein is introduced into the animal in solutions more acid than those in which it is isoelectric, it is a distinctly more potent antigen than when introduced in the solution with which it is isoelectric or in more alkaline solutions. Sensitization with 5 and intoxication with 50 milligrams of ovalbumin gives acute and usually fatal anaphylaxis when the sensitization is obtained with the protein at P_H 2.0-2.5 regardless of the acidity of the intoxication dose. Sensitization with the same dose of the protein at P_H 4.7-4.8 or at 9.0-10.0, regardless of the form of the intoxicating dose, gives reactions which range from only barely perceptible anaphylaxis to shock which is evidenced by the usual paralysis but which is usually lacking in the respiratory syndrome, the "air hunger," and which is practically never fatal. With larger sensitizing doses of protein (50 milligrams) acute anaphylaxis (including the respiratory as well as the paralytic reaction) can be produced by the protein at any of the three hydrogen-ion concentrations studied. We have obtained entirely similar results in guinea pigs which were passively sensitized with equal doses of the sera of rabbits, themselves actively sensitized with the acid, isoelectric and alkaline solutions of ovalbumin. Further, we have obtained similar results by the method of passive sensitization with a plant protein, the crystalline globulin edestin (isoelectric point, P_H = 6.9). Here again the protein in acid solution is a more effective sensitizing agent than in approximately isoelectric or in alkaline solution. Its intoxicating potency apparently is not affected by the P_H of the solutions at the points tested (2.0-2.5; 6.2-7.0; 9.0-10.0).

It is the tendency in immunological literature to stress the parallelism between anaphylactic and precipitating antibodies in immune sera. Indeed some workers incline to the view that the two are identical (Coca,[3] 1920). We have conducted some titrations of precipitins in the rabbit sera which were used for the passive sensitization of guinea pigs. With ovalbumin, the precipitin titrations were highest in the serum (anti-"acid antigen")

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[3] Coca, A. F., "Hypersensitiveness," Tice's Practice of Medicine, pp. 107-198.

which conferred the most intense passive hypersensitiveness in
guinea pigs, and lowest in the serum (anti-"alkaline antigen")
which conferred the lowest hypersensitiveness. With edestin the
precipitin titrations were reversed. The rabbit serum prepared
with "acid antigen," although more effective in sensitizing guinea
pigs, gave lower precipitin titrations than either of the sera pre-
pared against "unadjusted" or "alkaline antigen." The indica-
tions from these experiments are that modifications in the hydro-
gen-ion concentration may simultaneously affect anaphylactogenic
and precipitinogenic potencies of different proteins differently.
Some of the questions raised by these observations are being
studied further.

99 (2059)

Experiments on vitamin A.

By H. C. SHERMAN and M. M. KRAMER.

[From the Department of Chemistry, Columbia University,
New York City.]

These experiments relate chiefly to questions concerning the
storage of vitamin A in the body and the bearing of this upon
methods of examining foods to determine their relative richness
in vitamin A.

Even at weaning time young animals may already have a
considerable store of vitamin A in the body and thus be able to
continue to grow for some time upon a diet carefully freed from
vitamin A but adequate in all other respects. Young rats sepa-
rated from their mothers at a uniform "weaning" age of four
weeks show very different growth curves and survival periods
on the same experimental diet free from vitamin A, according
to the vitamin A content of the mother's diet. The differing
stores of vitamin A in the bodies of experimental animals, even
at early ages, has undoubtedly been a very large factor, not fully
appreciated, in previous experiments dealing with this vitamin

and in attempts to determine the vitamin A content of different foods.

The body can also store vitamin A at later ages. Thus two male rats of the same litter and of the same weight at four weeks of age were placed on two diets one of which was richer in vitamin A because of containing a higher proportion of milk than the other. After completion of growth, both were placed at the same time upon the same experimental diet deficient in vitamin A, and the survival period was found to be nearly twice as long in the case of the one which had received the diet containing the larger proportion of milk.

Rats of the same litter grown to adult size on the same diet, showed strikingly uniform survival periods when placed at the same age on diets devoid of vitamin A, even though these latter experimental diets were made to vary widely in their mineral content.

The investigation is being continued in several directions: (1) by further experiments with rats transferred at different ages from adequate diet to diet devoid of vitamin A, (2) by experiments in which the vitamin stored in different organs and tissues is determined directly by dissecting animals of known age and dietary history and feeding the different parts, (3) by further studies of possible relationships between vitamin A and the mineral metabolism.

100 (2060)

Some modifications of Emil Fisher's micro-polarimeter.

By SOMA WEISS

[*From the Department of Pharmacology, Cornell University Medical College, New York City.*]

The quantitative determination of organic compounds by means of their optical activity is often convenient, and it is often necessary to employ small amounts of the solutions to be investigated.

Emil Fischer's micro-polarimeter[1] has been used but very little, and it became impossible to follow his technique because of the inability to obtain the Nernst light as a consequence of the war, but I have applied the principle with several modifications, while using the Schmidt & Haensch polariscope, which permits of readings of 0.01 degree, and specially prepared capillary tubes which are 50 millimeters, and 100 millimeters in length, respectively, and which have an inside diameter of about 1.5 millimeters. The walls of the tubes are 3 mm. in thickness.

It is especially necessary that the tubes be perfectly straight. They hold 0.1 c.c. and 0.2 c.c. respectively, of the solution to be tested, the specific gravity of which is determined by means of a pycnometer holding 0.2 c.c.

The following method was used for the source of light: To a solution of 1.25 Gm. of pure uranium sulphate in 50 c.c. of distilled water was added 0.5 Gm. zinc powder and 1.8 c.c. of sulphuric acid, in three portions. After four hours, when the reaction was complete with the conversion of the uranyl sulphate into the uranosulphate, 2 cm. in length, the solution was filtered and the anterior cell of the polarimeter was filled with this solution completely avoiding air bubbles. The posterior cell, 2 cm. in length, was filled with a six per cent. solution of potassium bichromate. An electric lamp of opalescent glass of 150 watts intensity was placed in a galvanized iron casing into the side of which was fitted a telescope tube, permitting of variation in the distance of the light, and into which the end of the polariscope projected. This arrangement avoids the disturbance due to external light.

The capillary tubes were not encased in hard rubber, since that interferes with the observation of air bubbles during filling, but after filling they were placed in a previously slit rubber tubing. It was then found that the light which penetrated the glass walls of the tubes introduced a disturbing factor and this was avoided by covering the ends of the capillary tube with black paper which had an opening corresponding to the inside diameter of the tube. This arrangement insured that only that light which passed through the solution in the capillary tube passed on to the eye piece of the polarimeter.

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[1] *Ber. d. deut. chem. Gesell.*, 1911, xliv, 129.

A luer syringe, graduated in hundredths of a c.c., with a blunt needle corresponding to the lumen of the capillary tube, serves very well for filling the tubes, which are held nearly perpendicular while filling, thus avoiding the entrance of air.

Successive readings did not vary more than 0.03 degree and were often not more than 0.01 degree apart. The further technic and the calculations are carried out in the usual way.

The availability of the method is shown by the following results of a determination in which this method was employed:

Saccharose solution percentage.	Sp. Gr.	Rotation read in:		Spec. rotation in:	
		50 mm. tube	100 mm.	50 mm.	100 mm.
4.839	1.021	1.66°	3.32°	67.18	67.18
4.860	1.022	1.78	3.36	66.84	67.20
5.360	1.026	1.95	3.68	66.61	66.90
9.140	1.036	3.18	6.34	67.24	67.10
10.120	1.038	3.52	7.01	67.00	66.70

The table gives an average of +66.98 instead of +66.73, the theoretical value, for a 5 per cent. solution, and +67.00 instead of +66.65, the theoretical value for a 10 per cent. solution.

101 (2061)

Studies on the physiology of the parathyroids.

By HARALD A. SALVESEN (by invitation).

[*From the Physiological Institute of the University of Christiania, Norway.*]

We possess at the present time no exact knowledge concerning the function of the parathyroids. There are many theories. but only three have gained some importance, namely that the parathyroids control (1) the guanidine metabolism and by doing so regulate the tonus of the muscles; (2) the calcium metabolism; (3) the acid-base equilibrium and their removal cause alkalosis. The parathyroids also are supposed to control the sugar metabolism to some extent.

In the present work the changes in certain constituents of the blood have been studied following partial and complete parathyroidectomy in dogs, care being taken to leave enough thyroid tissue to prevent cachexia. Partial extirpation was done in 7 dogs, complete removal in 10 dogs.

I. Partial Parathyroidectomy. Three glands were removed. In no case did the animals show any symptoms of tetany, and apart from a little depression and loss of appetite the first few days showed no symptoms at all. In 6 of these dogs the fourth gland was removed later.

The blood sugar was absolutely unchanged. The alkali reserve usually was lowered the first few days after the operation; corresponding to this lowered alkali reserve there was an increase of acid elimination. The serum calcium decreased from a normal value of 10.0 mg. to a minimum of 7 mg. per 100 c.c.

In 2 of the 7 dogs there was no decrease in calcium; microscopic examination showed that only two parathyroids were taken out; in one of these dogs the third parathyroid was removed some time afterwards and the calcium dropped down to about 8 mg. Within two weeks the calcium was restored again to the normal. As a control a normal dog was given the same anesthetics used in the operations (chloroform and ether, equal parts); the alkali reserve dropped considerably, but the calcium remained unchanged. Another normal dog was given large doses of N/10 hydrochloric acid for three weeks; there was a heavy drop of the alkali reserve, but the calcium was unchanged. The calcium decrease found in partial parathyroidectomized dogs therefore cannot be due to anesthetics or acidosis.

II. Complete Parathyroidectomy. In 10 dogs complete parathyroidectomy was performed; in 6 of these partial parathyroidectomy was done previously, while in 4 all the parathyroids were removed at once. All of these 10 dogs developed acute tetany. Five of them died untreated within 3½ days; 1 died 22 days after; the condition was complicated by extensive necrosis of 3 legs following unsuccessful intravenous injections of calcium chloride. Four of these dogs were preserved by treatment and passed into a state of latent tetany.

A. Acute Tetany.

1. Blood analysis during development of the symptoms in this acute stage showed: Blood sugar unchanged; there was no

hypoglycemia even during the most acute symptoms shortly before death. The alkali reserve almost invariably decreased after the operation; it might be normal even during tetany. Corresponding to this drop of the alkali reserve there was an increase in the excretion of acids and there was no evidence of alkalosis, even close before the very onset of tetany. Calcium dropped from the normal value of 10 mg. per 100 c.c. serum and was, when tetany occurred, always below 7 mg. usually much lower. There seemed in this acute stage to be a certain relation between the degree of the lowering of calcium and the violence of the symptoms. The inorganic phosphorus of the blood serum increased considerably. After the initial rise the phosphorus might drop again, but still seemed to be decidedly above the normal value.

2. The Fate of Intravenously Injected Calcium Chloride in Parathyroidectomized Dogs.

One of the reasons why the decrease in calcium found in tetany is not regarded as the essential cause of the symptoms of parathyroidectomized dogs is, that they die in spite of calcium injections. This may be due to rapid elimination or carrying away of the injected calcium in some manner. The doses used by MacCallum and Voegtlin and others have been relatively small. In the present experiments 3 dogs were treated intravenously with a 10 per cent. solution of calcium chloride. A total of 2 grams a day was injected. This checked the symptoms, but almost invariably there was violent tetany again the next day, which was checked by a new injection. Such doeses of calcium *must* raise the calcium content of the body fluids considerably, and it is easy to calculate *how much* it will be raised knowing the value of blood calcium before injection, the weight of the dog and assuming an even distribution. Now, whenever convulsions reappeared after injection, the serum calcium always was found to be down to the same low level again, which shows that the injected calcium is disposed of in some manner.

To clear up this question 3 of the tetanic dogs were given a fixed dose of calcium chloride intravenously and the urine and feces for 24 hours were analyzed for calcium. The curves for the serum calcium and phosphorus were determined during this period.

TABLE I.

Dog II. Calcium and Phosphorus in Serum Following Injection of 1.6 gm.
Calcium Chloride Intravenously. Weight 14.5 kg.

Time.	Ca	P (inorg.).	Remarks.
	in 100 c.c. of serum.		
	mg.	*mg.*	
June 20			11:15 a.m., injection of 16 c.c.
before	5.2	7.9	10 per cent. sol. of $CaCl_2$.
11:30 a.m.	15.8	7.9	
2:00 p.m.	10.0	7.8	
3:30 p.m.	7.2	7.9	
8:00 p.m.	6.6	8.3	
12:00 a.m.	5.8	5.4	
June 21			
11:15 a.m.	5.3	6.7	

Table I shows that 15 minutes after the injection the calcium
was 15.8 mg. and decreased rapidly; 24 hours after, the calcium
had the same value as before injection; that is, there had disap-
peared from the blood an amount of calcium exactly correspond-
ing to the amount injected. Assuming an even distribution, the
injected calcium would increase the serum calcium to 10.9 mg.;
it will be seen that this level is not reached until about 2½ hours
later; which means that calcium chloride diffuses slowly from
the blood. There was also a rise in the phosphates.

TABLE II.

Dog II. Calcium Excretion in Urine and Feces, June 20-21, 1922.

Urine calcium.	Feces calcium.	Total.
g.	*g.*	*g.*
0.042	0.505	0.546
Injected		0.578

As Table II shows there was excreted an amount of calcium
corresponding to the amount injected, but more than 9/10 was
excreted in the feces and less than 1/10 in the urine (the colon
was rinsed out at the beginning and close of the period). This
is remarkable, as the calcium in the blood for some time must
have been decidedly above the threshold and still only small
amounts passed through the kidneys. This rapid disappearance
of calcium may explain why calcium treatment reported by pre-
vious workers failed to keep the animals alive.

*B. The Preservation of Completely Parathyroidectomized Dogs.
     Latent Tetany.*

Four of the completely parathyroidectomized dogs survived;
2 of them recovered spontaneously during milk feeding; the
other 2 recovered following vigorous calcium treatment com-
bined with milk feeding. In these latter dogs after a week or
two the doses employed (usually 1 or 2 grams of calcium chloride
a day intravenously) could be lowered and at last omitted; the
dogs lived without any symptoms and were to all appearance
normal on a milk diet. These 4 dogs could be kept alive inde-
finitely on this diet; one lived for 21 months and was then killed;
the others were either killed or at will brought into tetany from
which they died. These dogs which appeared absolutely normal
could, whenever it was wanted, be brought into tetany, usually
within 24 hours, by changing the diet to meat. This condition
formed an excellent opportunity for studying the various factors
involved in the production of tetany. Though to all appearances
normal these dogs showed one characteristic finding in the blood:
The calcium was still low. It varied to some extent, but was
usually between 5 and 6 mg. The inorganic phosphorus was
usually at the upper border of the normal level or above. Numer-
ous experiments have been performed on these dogs.

1. *The Diet in latent Tetany.* On a milk diet (minimum 500
c.c. a day with bread or porridge) these dogs after the first criti-
cal period was passed could be kept without symptoms for as
long a time as wanted. Meat always produced tetany, loss of
appetite, and depression. Prolonged meat feeding killed two of
the dogs. Experiments with various forms of diet showed that it
was the withdrawal of milk which produced tetany, not meat diet
in itself, and the conclusion is, that milk contains substances which
prevent tetany. This substance proved to be calcium of which
milk contains 1.2 grams per liter, corresponding to about 10
grams of calcium lactate. Ninety per cent. of this calcium was
precipitated by sodium oxalate, care being taken not to get any
excess of oxalate in the milk, and this milk was now useless in
preventing tetany; usually within 24 hours the dogs got violent
tetany when fed this milk.

2. *Calcium Administration.* On the other hand, if an amount
of soluble calcium salt was given corresponding to the Ca in the

amount of milk, which prevents tetany, the dogs kept normal on any diet; now when meat was given in excess, the dogs increased in weight and were normal in all their actions. It was possible by giving large amounts of calcium lactate by stomach tube to restore the calcium content of the blood almost to the normal level. From this condition it took a longer time than usual to produce tetany on a milk free diet and when tetany occurred serum calcium was always found lowered again. If tetany was produced from the latent stage a single dose of calcium lactate (5-10 grams) usually checked all the symptoms. Analysis of serum calcium then always showed that at the moment, when the symptoms were relieved, there was an increase in the calcium content of the blood, showing that it is the actual absorption of calcium which cures the symptoms. The same increase in serum calcium was seen when milk checked the symptoms. Concerning the relation between the blood calcium and phosphorus during latent tetany and the clinical symptoms, the findings may be summed up as follows: (a.) Whenever the animals were brought into tetany from a symptom free condition, the blood calcium was always still further lowered; (b.) When the animals by administration of calcium or milk were made normal again, the blood calcium was always higher than during the symptoms of insufficiency. (c.) The level of blood calcium at which tetany occurred seemed to vary and seemed to depend upon at what level of blood calcium the tetany producing diet was started. Inorganic phosphorus showed no regularity; it usually was higher than normal.

3. The protein metabolism in dogs with latent tetany on a milk diet and without symptoms was normal. The non protein nitrogen and blood urea were normal both in the latent state and during tetany.

4. Carbohydrate metabolism. The blood sugar was unchanged in these dogs both during the latent stage and during tetany. The tolerance for glucose given orally was lowered; when calcium in the blood was increased by calcium feeding, the tolerance was distinctly increased. When the dogs were brought into tetany by milk free diet the tolerance was lowered. The urine in one experiment contained 10 per cent. of sugar during the test. The blood sugar curves indicate that the lowered tolerance is not due

to increased permeability of the kidneys, but to a functional disturbance of the glycogen-forming organs. The results indicate that there is a relation between sugar tolerance and blood calcium in these animals.

5.   Guanidin injections in a normal dog in amounts large enough to produce violent convulsions did not alter the serum calcium but produced hyperglycemia and lowering of the alkali reserve; phosphates remained normal.

These experiments show that the characteristic feature in the chemistry of parathyroid insufficiency is the drop in blood calcium, which is more marked the more parathyroid tissue is removed. They suggest that the parathyroids control the calcium metabolism and by doing so they influence the function not only of the muscle and nerve tissue, but probably of all organs. When the parathyroids are removed, the threshold for the excretion of calcium in the intestines is lowered. The behavior of the blood calcium indicates that the actual recovery of completely parathyroidectomized dogs in these experiments is not due to compensatory hypertrophy of accessory glands, as the action of these would have been to restore the blood calcium to the normal level; the "adaptation" to a low calcium level, which in the beginning causes tetany, is not explained.

102 (2062)

Experimental diabète gras.

By G. A. FRIEDMAN and J. GOTTESMAN.

[From the Department of Clinical Pathology, College of Physicians and Surgeons, Columbia University, New York City]

French authors, especially Lancereaux,[1] have described two types of diabetes mellitus: diabète maigre and diabète gras. Lancereaux believed that the former was due to pancreatic dis-

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[1] Lancereaux, quoted by E. L. Opie, Diseases of the Pancreas, 1903, p. 308.

ease; diabetes with obesity, according to him, was not of pancreatic origin. Although most clinicians maintain that it is impossible to recognize the sharp distinction between diabète maigre and diabète gras, yet the fact remains that the physician in the majority of cases sees either lean or stout diabetics.

Diabetic children and young adults are usually lean, from the beginning of the disease until exitus. According to Joslin,[2] obesity is a marked feature of diabètes in the fifth and sixth decade of life. One must admit that while diabète gras may ultimately turn into diabète maigre, the latter condition rarely is transformed to the former.

Clinically three varieties of diabète gras may be distinguished:

1.   Obesity associated with excretion of sugar in the urine.

2.   Obesity with hyperglycemia in the absence of glycosuria.

3.   Obesity with lowered glucose tolerance in the absence of hyperglycemia or glycosuria.

The recognition of the first variety is simple. Diabetics belonging to the first variety may remain obese until coma sets in. In order to diagnose the second variety, one must be sure that the renal filter is intact. Under anti-diabetic treatment patients belonging to the second variety may temporarily lose in weight, the glycemia may become normal, but glucose tolerance is usually lowered.

It is our object to show that obesity and hyperglycemia can be produced in dogs in two ways:

1.   By almost complete thyroidectomy in partially depancreatized dogs.

2.   By ligation of one pancreatic duct.

It was previously shown that in persistently glycosuric dogs, after pancreatectomy, the glycosuria and hyperglycemia ceased after removal of the thyroid in toto, although the animals were on a liberal diet. The hyperglycemia, however, cannot be checked by slight suppression of thyroid function, as by partial ligation of the thyroid arteries, or by one-sided lobectomy. After these operations depancreatized dogs usually continue to lose weight as without these procedures. But if after removal of one lobe the second lobe of the thyroid is at a later date removed and a

2 Joslin, E. P., *Jour. Am. Med. Assn.*, 1921, lxxvi, 2, p. 79.

small fragment of thyroid tissue left behind in connection with the superior parathyroid, the animals gain considerably in weight although the hyperglycemia persists. The gain in body weight is also marked in depancreatized hyperglycemic dogs when incomplete thyroidectomy is done in one sitting. Such animals may live indefinitely and if they are disposed of with chloroform in from 82 to 128 days after almost complete removal of the thyroid, the striking finding at autopsies is: the thick layer of adipose tissue on the abdominal walls and in the omentum. The thyroid remnants in these dogs were found to be hypertrophied.

As to the second mode of production of diabète gras, it is well known from the experiments of Ssobolew[3] and especially from the experiments of Banting[4] and Best, that from 8 to 10 weeks after ligation of the pancreatic ducts, the digestive apparatus of the pancreas degenerates and the Langerhans islands remain intact. The blood sugar was never increased after this procedure in the experiments of the latter authors. We found that when one duct of the pancrease was ligated, the animals showed increased bloodsugar content similar to partially depancreatized dogs. These dogs also gained considerably in weight, remained in excellent condition and when they were killed 28 to 42 days after ligation, the findings in the abdominal walls and omentum were similar to those in the obese dogs after incomplete thyroidectomy. Inasmuch as at the former laparatomies no abundance of fat was noted, the adiposity must have been due to the procedure on the thyroid or to partial duct ligation.

Although we admit that our material consists of only eight dogs in whom obesity and hyperglycemia were marked features, we believe we are justified in presenting this work on account of the uniformity of results. The animals during life resembled human beings in the preglycosuric state when they are obese and in the absence of renal disorders show hyperglycemia or lowered glucose tolerance. In concluding it may be said that since Banting and Best have discovered the pancreatic hormone, there hardly remains a doubt as to the pancreatic origin of milder forms of diabetes,—diabète gras of the French.

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3 Ssobolow, L. W., *Wirch. Arch.*, 1902, clxviii, 91.

4 Banting, F. G., and Best, C. H., *J. Lab. and Clin. Med.*, 1922, vii, 251.

103 (2063)

The thyroid factor in diabète gras.

By G. A. FRIEDMAN and J. GOTTESMAN.

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The fact that diabète gras can be produced by suppressing thyroid function through almost complete removal of the thyroid, points to the share of thyroid deficiency in mild diabétes. This assumption is strengthened by the fact that all thyroids in the dogs after partial duct ligation showed marked atrophic areas on microscopical examination.

It seems then that obesity does not predispose to diabètes as it is generally believed, but that obesity results from mild diabetes, as in middle aged patients. If general adiposity would be the predisposing factor in diabètes, one cannot see why it should affect the Langerhans islands, leaving intact the secreting acini of the pancreas. Since the external secretory apparatus must also become affected by the fat deposit, symptoms or signs of a disturbance in the digestive pancreatic apparatus must establish itself in genuine diabetes. It is a fact that pancreatic acini remain intact in diabetes mellitus. The clinician at least is not aware of a disturbance in the latter.

Another argument: If obesity predisposes to diabetes, a lowered glucose tolerance should frequently be found in obese persons. Paullin[1] made glucose tolerance tests in 26 cases of obesity without renal disorders. Five of these patients showed a lowered sugar tolerance and two of them later actually developed glycosuria.

That mildness of diabetes varies directly with thyroid dysfunction may be concluded from the experiments of Wilder[2] and his coworkers. Throughout these experiments in human diabetes the glucose tolerance varied inversely with the basal metabolism. The tolerance rose when the metabolic rate fell and fell when the

1 Paullin, J. E., *J. Am. Med. Assn.*, 1921, lxxvii, 1996.
2 Wilder, R., Boothby, W. M., and Beeler, C., *J. Biol. Chemist.*, 1922, li, 311.

rate rose.   Furthermore, throughout all their experiments the basal metabolic rate varied with the general condition of the patient.   When the rates were lowest the glucose utilization was best and acidosis was either controlled or decreased.   When the basal metabolic rate rose sugar tolerance was diminished and acidosis increased.

ABSTRACTS OF COMMUNICATIONS, PEKING BRANCH

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104 (2064)

Some experimental observations on the retina of the gecko.

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The association of visual purple or rhodopsin with the visual function of the rods in dim or twilight vision as maintained in the duplicity theory of von Kries (Nagel,[1] Helmholtz,[2] Parsons,[3] and Hartridge[4]) is generally regarded as well substantiated.

As to the genesis of this pigment, numerous ideas have been advanced, although it is generally held that it is a product of the epithelial pigment cell (Garten[5] and Kolmer[6]).

In the present work on the gecko retina, which is cone-free,

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1 Nagel, W., Handbuch der Physiologie des Menchen, Bd. 3, 1905.

2 Helmholtz, H. von, Handbuch der physiologischen Optik, Bd. 2, 1911.

3 Parsons, J. H., An Introduction to the Study of Colour Vision, 1915.

4 Hartridge, H., ''Vision,'' pp. 486-588, Starling's Human Physiology, 1920.

5 Garten, S., Graefe-Saemisch Handbuch der gesammten Augenheilkunde, 1907, Bd. 3, Kap. 12, 130 pp.

6 Kolmer, W., *Pflüger's Archiv.*, 1909, cxxix, 35.

certain observations have been made which suggest that the outer segment of the rod may be more directly concerned in the production of this pigment.

When the retina is preserved in modified Held's fixative (v. Kolmer, op. cit.) and stained with iron haematoxylin and erythrosin, numerous droplets, which stain with a varying intensity from gray to black, are seen on and between the outer segments of the rods. In addition, a characteristic lamellar arrangement of deeply staining granular material in the outer segment of the rod is very evident. When stained sections of eyes which have been kept in darkness for twenty-four hours are studied microscopically, the droplets are found to be very numerous and the deeply stained granular material in the rod outer segment is greatly reduced in amount or is not to be seen at all. On the other hand, identically prepared sections of eyes that have been exposed to light for six hours not only show a great reduction in the number of droplets, but the granular striations in the rods are very compact and deeply stained. Intergrades between the two extreme conditions are also to be found and, within the same retina, whether light or dark adapted, there is a stiking correlation between the number of droplets present and the amount of granular material within the rod outer segment.

Kolmer described the presence of these droplets in the retinæ of various vertebrates. In the frog retina kept in darkness, he found these droplets to be larger and more numerous than in the illuminated retina, and after illumination with direct sunlight, they were not to be seen at all. These droplets are ascribed by Kolmer to be secretion products of the retinal epithelial cells and he makes no mention of a relationship existing between the number of droplets and the amount of granular material in the rod outer segment. His failure to find them in the pure cone retinæ of lizards and snakes, strengthened his assumption that they are concerned in rod vision and perhaps with the appearance of visual purple.

From the study of these droplets in the gecko retina, two significant points are brought out. The number of droplets outside the rod seems to be inversely proportional to the amount of granular striated material within the rod outer segment. Their genesis from the rod, therefore, seems to be less questionable than an origin from the pigment epithelial cells. Further, since

they are found to be more numerous in the dark-adapted retina than in the light-adapted one, the extent of their presence is more or less in correspondence with the conditions responsible for the regeneration and the bleaching of visual purple.

When sections of light-adapted eyes of field mice are compared with sections of dark-adapted ones, the droplets occur in considerably reduced numbers in the sections of the light eye as compared with those in the dark eye; and the histological comparison corresponds closely with that found in the gecko eye under the same conditions. However, a comparative study of light and dark-adapted eyes of Eremias (a diurnal lizard possessing cones only) shows a total absence of these droplets under both conditions.

The exclusive presence of these droplets in rod retinæ, as well as the difference which exists between eyes kept in darkness and those exposed to light, is highly suggestive that they are concerned with the appearance and disappearance of visual purple under the two respective conditions, although it would be premature to say definitely that they represent a histological picture of this pigment.

A more complete account of these experiments and of additional ones will be forthcoming.

The intermediate host of schistosoma japonicum in China.

105 (2065)

By HENRY EDMUND MELENEY, and ERNEST CARROLL FAUST.

[From the Parasitology Laboratory, Department of Pathology, Peking Union Medical College, Peking, China.]

The snail which acts as the intermediate host of *Schistosoma japonicum* in Japan has been known since 1913 to be *Blanfordia nosophora*. No intermediate host for the parasite has heretofore been described for China. We selected the endemic region about Soochow, in Kiangsu Province, for our search. Our method

consisted of first finding a case of the disease and then hunting in the neighborhood of the patient's home for a snail somewhat similar to *Blanfordia nosophora*. On August 11, 1922, such a snail was found in large numbers on the shore of a small terminal canal near which many cases of schistosomiasis japonica resided. Examination of these snails by crushing their shells revealed a five per cent. infection with cercariae morphologically similar to that of *Schistosoma japonicum*. A mouse immersed in water containing these cercariae was found, thirty-one days later, to harbor adult forms of *Schistosoma japonicum* in the portal and mesenteric veins. Snails of this type were exposed to miracidia of *Schistosoma japonicum* and at intervals thereafter were killed, and showed in serial section the development of the sporocyst and cercaria forms of the parasite.

The snail which acts as the intermediate host of *Schistosoma japonicum* in the Soochow district is an operculate gasteropod averaging 6.4 mm. in length by 3.0 mm. in breadth. Its shell is acuminate in form, has seven spirals, is dextrally coiled, is of dense consistency and has closely set ridges running perpendicular to the spiral groove of the shell. Its shell differs from that of *Blanfordia nosophora* in its greater density, its ribbed surface and its greater breadth in proportion to its length. These features place the Chinese intermediate host in a different genus from the Japanese host. Owing to the difficulty of correctly classifying the members of the genus to which this snail apparently belongs, we have referred it for classification to an expert malacologist, Mr. Bryant Walker of Detroit.

The snail was found in the Soochow district on the shore of small canals, particularly terminal ones. It was usually found just above the water's edge, and in fewer numbers just beneath the surface of the water. It preferred secluded spots protected from the sun. It was not found in the rice fields, probably because in this district the fields are from four to six feet above the level of the canals, and are therefore too dry for the snail's existence.

In order to check up by biological phenomena the morphological similarity of the forms of *Schistosoma japonicum* developed from Japanese and Chinese material, we exposed the Japanese intermediate host to miracidia from a Chinese patient, and the Chinese intermediate host to miracidia from a dog infected with

cercariae obtained from specimens of *Blanfordia nosophora* from Japan. In both cases serial microscopic sections of snails so exposed showed the successful penetration of the snail by the miracidia. The biological similarity of the Japanese and Chinese forms of *Schistosoma japonicum* is therefore demonstrated.

106 (2066)

Factors controlling the electrolyte and water distribution in the blood.

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[*From the Laboratory of Physiological Chemistry and the Chemical Laboratory of the Department of Medicine, Peking Union Medical College, Peking, China.*]

In combining the known facts to form an inclusive quantitative expression of the phenomena of electrolyte and water distribution we have assumed for the blood the validity of the following physico-chemical laws:

I. At and near the neutral point all strong alkalies are in the form of salts. At blood reaction therefore the total base may be represented as $BP + BA$, when BP represents the alkali protein salts, BA the salts bound by the alkali with other negative radicles, chiefly Cl' and HCO_3'.

II. The law of Donnan governing the influence of non-permeating ions on the distribution of permeating ions on the two sides of a membrane holds for the membranes of the blood cells. Donnan's theory has been provided with a sound basis of experimental proof by Donnan, by Proctor and Wilson, and especially by Loeb's recent study on the osomotic and electrical behaviors of protein solutions.

III. The osmotic activity of each solute is proportional to the ratio $n : N$, of gram molecules of solute to gram molecules of water. The presence of the serum proteins, according to the vapor tension determinations of Neuhausen, does not affect the

validity of this ratio as the governing factor of osmotic activity.

On the basis of the assumption that the above laws are valid for blood, mathematical expressions have been derived which indicate the distribution of electrolytes and water between cells and serum, and the manner in which the distribution is affected by changes in P_H (CO_2 tension) and oxygen content. The effect of varying CO_2 tensions has been investigated, and the results have been found to accord with those calculated on the basis of the above assumption.

107 (2067)

Leishmania donovani in the peripheral blood.

By CHARLES W. YOUNG and HELEN M. VAN SANT.

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Donovan[1] who found *Leishmania donovani* first in the living patient also discovered the parasite in smears from the peripheral blood. Many have advocated the examination stained blood smears for diagnosis of the disease (Patton,[2] Marshall,[3] Cannata,[4] Knowles[5]) but all agree that the organism is present in small numbers and that many slides must be carefully studied. Spleen smears obtained by aspiration has been the usual method for diagnosis. As the bleeding time is prolonged in all advanced cases this procedure is not devoid of danger (Donovan,[1] Wylie,[6] Knowles,[7] Rogers,[8] Bramachari[9]). Blood culture has been suggested as a substitute but results have been inconstant (Mayer and Werner,[10] Wenyon,[11] Row,[12] Korke,[13] Knowles[5]). The last named author made 128 cultures from 34 patients with two posi-

1 Donovan, *Lancet*, 1904, ii, 744.

2 Patton, W. S., *Indian Jour. Med. Res.*, 1914, ii, 492.

3 Marshall, W. E., Fourth Report, Wellcome Trop. Res. Labs., Khartoum, 1911, 157.

4 Cannata, S., quoted by Wenyon, *Trop. Dis. Bull.*, 1922, xix, 1.

5 Knowles, R., *Indian Jour. Med. Res.*, 1920, viii, 162.

tive cultures from one patient. At first Rogers merely citrated the patient's blood but Nicolle was much more successful with his "N.N.N." medium, a rabbit blood agar. The experiments of Rogers[15] and of Cornwall and LaFrenais[14] showed that culture media made with human blood were not favorable to the growth of *Leishmania donovani*.

Two series of experiments were made using salt agar as in N.N.N. medium but with washed red cells and serum, both unheated and heated at 56 C° for one-half hour, from man, horse, sheep and rabbit. Human red cells were somewhat inhibitory; human serum much more so. Rabbit cells and serum were less unfavorable to growth but were inferior to whole defibinated rabbit blood. In action the cells and serum of the horse and sheep lay between those of man and the rabbit. As a result of these experiments, a technic for blood culture was devised as follows: 10 c.c. of blood was drawn from a vein into 2 c.c. of 1 per cent. citrated Locke's solution in a syringe and immediately expelled into 50-70 c.c. of the same fluid in a flask. On return to the laboratory, the diluted blood was divided between two 50 c.c. centrifuge tubes and spun slowly (about 750 r.p.m.) for five minutes. The blood was thus divided into a supernatant cloudy fluid and a sediment of red cells. The cloudiness of the first is caused by blood platelets and some red cells. Extracellular Leishman-Donovan bodies were occasionally found in this fraction. After rapid centrifugation (about 1400 r.p.m. for five minutes) the sediment from this upper portion was planted into N.N.N. medium of about P_H 7.6. Using this technic, 115 culture tubes were made from 34 blood specimens from untreated cases of kala-

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6 Wylie, J. H., *China Med. Jour.*, 1920, xxxiv, 593.

7 Knowles, R., *Indian Jour. Med. Res.*, 1920, viii, 177.

8 Rogers, Sir Leonard, Fevers in the Tropics, Third Ed., p. 48.

9 Bramachari, A. N., Kala-azar and Its Treatment, Butterworth, 1920, p. 99.

10 Mayer, M., and Werner, H., *Deutsch. Med. Wchnschr.*, 1914, xl, 67.

11 Wenyon, C. M., *Jour. Trop. Med. and Hyg.*, 1914, xvii, 49.

12 Row, R., *Indian Jour. Med. Res.*, 1914, ii, July editorial.

13 Korke, V., noted in Cornwall and LaFrenais, *Indian Jour. Med. Res.*, 1915-16, iii, 398.

14 Cornwall, S., and LaFrenais, H. M., *Indian Jour. Med. Res.*, 1915-16, iii, 398.

15 Rogers, Sir Leonard, *Lancet*, 1919, i, 505.

azar.   Thirty-one of the specimens showed flagellates (91 per cent.), 82/115 culture tubes being positive.   134 cultures were also made from forty specimens of blood from kala-azar patients treated with several forms of antimony.   When these were arranged in ascending order of doses of milligrams of antimony per kilogram weight of patient it was found that up to a total dosage of 12 mg. (0.162 to 11.96 mg.) positive cultures were obtained in 60 per cent. of the fifteen cases and in 22/59 of the culture tubes.   Beyond that point, i. e., doses of 20.66 to 77.64 mg., seventy-five cultures from twenty-five specimens were uniformly negative.   Three similar series were made from spleen juice.   The first showed twenty out of twenty-two (91 per cent.) specimens positive (68/108 tubes) in untreated cases.   The nine specimens from patients treated up to 12 mg. (0.05 to 10.2 mg.) per kilogram of their weights had seven positive (77 per cent.) with 30/56 of the tubes showing growth.   Only three spleen cultures were made from patients who had received more than 12 mg. (12.9, 30.7 and 95.87 mg.)   All were negative.   Comparison was made betwen the results obtained by the author's technique (Series A) and that of Row[16] (Series B).   A third series (Series C) was made from the cloudy fluid above Row's sediment after centrifugation.   Series A showed 30/69 (43.5 per cent.) tubes positive; Series B, 16/46 (34.8 per cent.) and Series C 10/43 (23.3 per cent.)   The only obvious difference between Series A and C was that in the latter, the material had stood in contact with the diluted human serum for from 15 to 20 hours. The greater number of positive cultures in Series B than in Series C suggested a search of the layers of the red cell sediment obtained after slow centrifuging of the diluted blood specimen.

In the bottommost part of this sediment both mononuclear and polymorphonuclear leucocytes were found and in a few of them, Leishman-Donovan bodies.   Of twenty-seven smears made from nine blood specimens from five untreated kala-azar patients twenty-one showed intracellular parasites.   Of twenty-six slides from seven specimens from four cases treated with 0.49 to 5.13 mg. of antimony per kilogram of patient's weight fifteen gave

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16 Row diluted ½-2 c.c. of blood in 20 c.c. of citrated saline and after sedimentation over night, planted the sediment in N.N.N. tubes.

positive results (*i. e.,* 5/7 specimens positive). None of the seven blood specimens from six who had received 28.68 to 77.64 mg. of antimony per kilogram of their weights showed any Leishman-Donovan bodies. The method followed was to withdraw a column of blood 1 to 2 cm. high in a fine capillary pipette from the bottom of the conical tube in which the diluted blood specimen was centrifuged. The smears were made on slides in the usual manner. Wright's or Leishman's stain was used.

1. The effect of human red cells and serum on the growth of *Leishmania donovani* has been studied and a blood culture method devised, based on these findings.

2. The distribution of blood platelets and of the different varieties of leucocytes in the strata of centrifuged diluted blood has been investigated and also the distribution of Leishman-Donovan bodies, extracellular and intracellular in these strata.

3. The effect of treatment with antimony on the cultivability of *Leishmania donovani* has been studied.

Conclusions

1. Human red cells in a culture medium permit only a feeble growth of *Leishmania donovani.*

2. Human serum, whether inactivated or not is distinctly inhibitory to the growth of this parasite.

3. If an adequate amount of blood is taken (10 c.c.) and most of the serum and red cells removed by centrifugation, positive cultures may be obtained in about 90 per cent. of untrated cases of kala-azar.

4. By this technic positive cultures may be obtained as frequently from peripheral blood as from spleen punctures.

5. Extracellular Leishman-Donovan bodies are approximately of the size and specific gravity of platelets and are found in the "platelet fraction" of centrifuged diluted blood.

6. Mononuclear and polymorphonuclear leucocytes with the contained intracellular forms are usually heavier than the other blood cells and go to the bottom of the tube on centrifugation or sedimentation.

7. In the series of cultures made, the blood of patients treated intravenously with antimony salts became free from cultivable *Leishmania donovani* after they had received about twelve milligrams of antimony per kilogram of their body weights.

8. . Cultures from spleen punctures show similar results.

108 (2068)

A method for demonstrating growth-inhibitory and bactericidal action on the pneumococcus of a normal serum-leucocyte mixture.

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Methods heretofore employed in testing for growth inhibiting and bactericidal action of the blood on pneumococcus have consisted in suspending small numbers of pneumococci in whole blood, serum or serum-leucocyte mixtures, contained in the capillary pipette, test tube or hanging drop. Results of tests on serum and serum-leucocyte mixtures have shown a general agreement that neither serum nor serum and leucocytes together inhibit the growth of the pneumococcus. Studies on whole blood, however, have resulted in most divergent findings. Certain workers (Wright, Heist and Solis Cohen) report the finding not only of growth inhibition, but also of pneumococcidal activity in the blood of animals resistant to pneumococcus infection. Other investigators, (Barber, Bull and Bartual) using the same methods on the blood of the same and other resistant animal species failed to find anything more than growth retardation. A review of the more important literature on this subject leads one to the conclusion that with the methods heretofore employed it is not possible to demonstrate with any degree of constancy either growth inhibitory or bactericidal activity of the normal blood for the pneumococcus.

It seemed probable to the writers that further information on this subject could be obtained were it possible to work out a method that should incorporate certain conditions under which inhibition of growth with subsequent destruction of the pneumococcus might be expected to occur in the body. In the tissues of the animal body fluid currents operate to bring the leucocytes and implanted micro-organisms into intimate contact. In the capillary pipette or test tube this constant mixing process is absent. Growth inhibition and bacteriolysis probably occur only if

all the organisms come into contact with and are phagocyted by actively functioning leucocytes.

A method has been devised for carrying out growth inhibition tests with the pneumococcus by which a constant and thorough mixing of leucocytes and micro-organisms is obtained during incubation. For this purpose mixtures of serum and washed leucocytes placed in small glass tubes are seeded with varying numbers of pneumococci. All the constituents are added in known quantities. The tubes are then sealed with paraffined corks and attached to an agitating apparatus placed in the incubator.

The apparatus consists of two solid wheels made of wood and mounted on an axle which rests on a central pivot-bearing placed midway between the two wheels. The axle is made to oscillate in a vertical plane by means of an eccentric. A small motor supplies the power, the speed being reduced to any desired rate by means of a series of pulleys. A leather belt with a piece of tape run through a series of slits cut in the leather serves as a holder for the tubes. This is attached to the wheel. When the apparatus is in operation, the motion given to the tubes, rotation plus oscillation, serves to mix their contents thoroughly and at the same time keeps all parts of the inside surface of the tubes moistened. The importance of a continuous washing of the tubes' contents over all parts of the inside surface is readily seen since the occurrence of any drying would probably mean the escape of some of the organisms from phagocytosis. Rotation and oscillation are maintained at a slow rate so as to reduce to a minimum mechanical injury to the leucocytes.

The pneumococci used for the test were suspended in Locke's solution P_H 7.8-8.0, to which 0.125 per cent. of gelatin had been added for the purpose of better preserving the organisms. The suspension was standardized by means of Gates' turbidimeter method with preliminary bacterial counts. A standard suspension of approximately 1,000 million pneumococci was used. Dilutions were made from the suspension in gelatin Locke's solution. Only organisms in the active growth phase were employed in the tests. The strain used was of low virulence for the cat, but highly virulent for rabbits, guinea pigs and mice. The leucocytes were obtained from pleural exudate produced by the injection of aleuronat. The exudate was mixed in the pleural cavity with equal parts of 1 per cent. sodium citrate in normal salt solution. After

centrifugation the cells were suspended in gelatin salt solution and a count of the white blood cells made. A second washing with gelatin Locke's solution was done and the cells finally suspended in this solution in known concentration. Solutions for washing and suspending leucocytes were adjusted to P_H 7.2-7.6. In addition to the leucocytes washed red blood cells were added as indicators of pneumococcus growth.

Results

It was found that pneumococci seeded into 0.3 c.c. cat's serum plus 0.1 c.c. leucocyte suspension containing 50,000 white blood cells per c.mm. (the equivalent number of white blood cells contained in 0.5 c.c. of blood with a count of 10,000 per c.mm.) failed to grow in numbers less than 0.001 c.c., or at most 0.0001 c.c., whereas the control tubes containing serum alone showed growth with 0.0000001 c.c. of organisms. The tubes were allowed to incubate for varying lengths of time, from 24-72 hours before microscopic examination was made. The contents of those tubes showing no growth were transferred into 1 per cent. dextrose blood broth P_H 8.0 and rabbit blood agar plates P_H 7.8 in order to determine the presence or absence of living pneumococci. Organisms could not be recovered from the tubes which failed to show growth. The media used for these tests had been determined beforehand to be highly favorable for the growth of very small numbers of pneumococci. Further experiments were made in which mice were employed as a culture media. The results of these tests showed that after 24 hours sojourn in the cat serum-leucocyte mixture as much as 10,000 times the killing dose of pneumococci failed to kill the test mice. Tests carried out on the dog serum and leucocytes gave similar results. On the other hand, the serum-leucocyte mixtures of susceptible animals, the rabbit and guinea pig, showed no growth inhibiting action against pneumococci. Even such a small number of organisms as 0.0000001 c.c. of the standard suspension grew readily in the blood elements of these animals.

The results of the above experiments seem to warrant the conclusion that with the technique employed, a mixture of serum and leucocytes from resistant animals can be shown to exert not only a growth inhibiting but also a bactericidal action on the pneumococcus.

109 (2069)

The susceptibility of cells to radium radiations.

By CHARLES PACKARD.

[*From the Peking Union Medical College, Peking, China.*]

Among the various factors which influence the response of cells to radium radiations are (1) the temperature of the cells at the time of radiation, and (2) the permeability of the cell membrane. This statement is based on the results of experiments with paramoecium and some other protozoa. The radium, amounting to 13.4 mg of element, was enclosed in a thin-walled glass capsule, and was held at a distance of 2 mm from the water containing the cells. All the paramoecia used were descendants of a single wild cell.

Paramoecium cells are more susceptible to radiations at the upper limit of their physiological range of temperature than at their lower limit. At 14° C. the lethal dose is about 10 hours; at 37° C. it is 1½ hours. These temperatures in themselves are not injurious to the cells. For each rise of 8° C., the length of the lethal dose is halved. In lower temperatures the lethal dose is somewhat shorter than would be expected. The curve expressing these relations is the same as that which expresses the relation between temperature and the rate of metabolism in paramoecium as shown by the rapidity of cell division and of the pulsating of the contractile vacuole.

Cells whose membranes are relatively permeable are more susceptible to radiations than those whose membranes are relatively impermeable. The permeability of paramoecium and other protozoa is determined by the rate at which dilute NH_4OH enters the cells and decolorizes the neutral red in which they have been stained. At different periods of its life cycle, paramoecium shows varying permeability. During conjugation the cells destain in 7 minutes, when treated with $n/1500$ NH_4OH, while single cells destain in 20 minutes. At 21° C. the lethal dose of radiation for conjugating cells is 3½ hours: that of single cells is 5½ hours. A comparison of the permeability of paramoecium with that of Stylonichia shows that the former cells destain in about one-

fifth of the time required by the latter. The length of the lethal dose of radiations for these two forms is about in the same proportion.

It can be shown that one of the physical effects of the radiations is to increase the permeability of the cells by injuring the cell wall. If the treatment is long enough continued the cells cytolyze completely. From this it follows that when cells are already highly permeable, as they are during growth and division, complete cytolysis quickly ensues: whereas when the cell membrane is relatively impermeable, as it is in resting cells, radiation must be long continued before destructive cytolysis can be observed.

ABSTRACTS OF COMMUNICATIONS, MINNESOTA BRANCH

Ninth meeting.

Minneapolis, Minnesota, January 10, 1923

110 (2070)

The effect of increase of blood pressure on the concentration of colloidal dyes in the plasma.

By F. H. SCOTT, M. RABINOWITZ, and A. RUPP.

[From the Department of Physiology, University of Minnesota, Minneapolis, Minnesota.]

Great changes in the number or volume of the corpuscles, or in the haemoglobin content of the blood may occur in a very short time. In using corpuscles or their derivatives as the indicator of the concentration of the blood one meets with the objection that there might be hidden masses of corpuscles ready to be put into the circulation in times of stress. No evidence exists for such masses of corpuscles and it is well known that dilution of the blood may take place equally as rapidly as concentration. In the case of the dilution caused by haemorrhage

one of the authors[1] showed the fluid coming in from the tissue spaces was almost pure salt solution having a protein content of only 0.6 to 2 per cent. If changes in the concentration of the plasma could be shown when the blood becomes concentrated in corpuscles, it would get away from all objections of hidden masses of corpuscles.

In the experiments recorded here the concentration of injected congo red has been followed in the plasma. This method is not ideal for all phases of this problem because there is normally a gradual decrease in its concentration. Thus, according to Harris,[2] considering 100 as the percentage after the dye has been uniformly mixed with the plasma there is a fall of about 4 per cent. in five minutes. This is however, slower than with vital red. This figure is not fixed and sometimes we found larger figures than that and sometimes less. If, after this second sample of blood has been taken, the blood pressure be raised by the injection of adrenalin or by section of the vagi and a third sample taken after a few minutes, one finds the concentration of congo increased. Thus, in one instance nineteen minutes after injection of congo and fourteen minutes after the first sample of blood containing congo had been taken we have found 111 per cent. of congo in the plasma as a result of adrenalin. This was in an 18 kilo. dog. The dog had about 693 c.c. plasma to commence with and this would mean about 63 c.c. of fluid had left the blood provided no congo had left in the time. Because congo is leaving the blood all the time the method of following dyes can give only relatively crude figures, but they show such results as were obtained by one of the authors[3] when the haemoglobin change was followed, are due to fluid leaving the blood and not to hidden masses of corpuscles. They indicated that the colloid of the blood does not leave the blood as fast as the water. The changes in the concentration of the natural colloid of the blood are being investigated.

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[1] *Journal of Physiology*, 1916, 1, 157.
[2] *Brit. J. Exp. Path.*, i. 162.
[3] *American Journal of Physiology*, 1917, xliv, 298.

111 (2071)

The effect of wetting on the pathogenicity and viability of the tubercle bacillus.

By W. P. LARSON and IRWIN A. MONTANK.

[*From the Department of Bacteriology, University of Minnesota, Minneapolis, Minnesota.*]

In papers published from this laboratory during the past three years attention has been directed to changes produced in cultures of pellicle forming bacteria by adding surface tension depressants to the media. The changed form of growth from pellicle to diffuse growth thus induced has been attributed[1] to differences in degree of wetting of the bacteria.

We have found that the tubercle bacillus can be made to grow beneath the surface of glycerine broth by appropriately depressing the surface tension of the medium. The tubercle bacillus, like other pellicle forming organisms, we believe, grows on the surface because of its high fat content which tends to resist wetting. In a recent paper Larson and Larson[2] have shown that practically all aerobic bacteria may be grown on the surface of broth by growing them on media from which they may store up fat in increased amounts.

The present study concerns the effect of wetting on the viability and pathogenicity of the tubercle bacillus. A virulent culture of this organism was grown in glycerine broth to which had been added sufficient soap to depress the surface tension to 44 dynes. Growth under these conditions is under the surface of the medium and is slower than when grown under the usual conditions. Inoculation of such cultures subcutaneously into the flank of guinea pigs was followed by a slight enlargement of the inguinal glands of the side inoculated. Within the following ten days this glandular enlargement had receded, after which the animals showed no signs of tubercular infection.

The mechanism of this rapid loss of pathogenicity is difficult of analysis at the present time. However, if the hypothesis is

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[1] Larson, W. P., PROC. SOC. EXP. BIOL. AND MED., 1921, xix, 62-63.
[2] Larson and Larson, *Jour. Inf. Dis.*, 1922, xxxi, 407.

accepted that pellicle formation of the tubercle bacillus is an indication of minimum wetting, the same arguments may be used to explain altered pathogenicity. When the tubercle bacilli are growing diffusely throughout the medium they are, according to this hypothesis, wetted, and therefore when introduced into the animal body are penetrated by the antibodies or bactericidal substances present, and destroyed.

Calmette[3] has recently been able to deprive the tubercle bacillus of its pathogenicity by growing it in bile broth. Since bile is a surface tension depressant it is reasonable to assume that its action upon the growth of the tubercle bacillus is much the same as that of soap. Calmette does not state whether the amount of bile used in his medium caused the tubercle bacillus to grow diffusely through a liquid medium. We have found that when bile is added to glycerine broth so that the surface tension is approximately 44 dynes it grows beneath the surface as it does when soap is used. Growth in bile medium is more rapid and more profuse than in the soap broth. We are of the opinion that the attenuation of the tubercle bacillus, as observed by Calmette, is due to a wetting phenomenon.

We have also investigated the effect of sodium rescinoleate on the pathogenicity of tubercle bacilli in sputum. Equal amounts of T.B. sputum and 2 per cent. solution of sodium rescinoleate were mixed and allowed to stand for several hours. A quantity of the mixture was then injected subcutaneously into a series of guinea pigs. The controls were inoculated with half the volume of the untreated sputum. The animals inoculated with the soap-sputum mixture developed slight enlargement of the lymph glands draining the inoculated area. Fourteen days later the glands had returned to normal in all the animals, none of which developed further signs of tuberculosis.

The controls all died with the characteristic picture of experimental tuberculosis.

The question naturally arose as to whether the sodium rescinoleote had killed the tubercle bacilli or merely altered their pathogenicity. This problem was attacked by placing a small fragment of pelicle from a young culture on glycerine broth into a

3 Calmette, A., *Ann. de l'Inst. Pasteur. Par.*, 1921, xxxv, 561-570.

1 per cent. castor oil soap solution, and making cultures at given intervals. The organisms which were thus transferred from the soap solution to glycerine broth sank to the bottom of the flask immediately and there was no growth even after a prolonged period of incubation. If, however, the cultures were made on the surface of egg medium, or supported in the surface of glycerine broth by means of a wire gauze, growth developed with characteristic pellicle formation.

From this experiment we conclude that, under these experimental conditions, the pathogenic properties of the tubercle bacilli are lost before the property to reproduce. The fact that we obtained growth in the above experiment when the organisms were supported in the surface of the glycerine broth, and a negative culture when the inoculun fell to the bottom of the flask will be attributed by most bacteriologists as due to the influence of oxygen. Bacteriologists have for years considered pellicle growth as evidence of obligatory aerobiosis. But those who hold to this view have not offered an explanation as to the force which supports the organisms on the surface of a liquid medium.

With few exceptions most pellicles may be sedimented by slight agitation or centrifugation.

This indicates clearly that they are of higher specific gravity than the medium upon which they are growing. It follows therefore that they are supported on the surface of a fluid medium by some force. Since "demand for oxygen" is not a force it follows further that they are not on the surface because of their demand for oxygen, although the oxygen present may, undoubtedly does, influence their development. The fact that tubercle bacilli will grow diffusely through the medium by appropriately depressing its surface tension is evidence that it will grow under conditions where the oxygen tension is not at its maximum. Its tendency to grow only in or upon the surface of glycerine broth is probably due to the concentration of surface tension depressants there, which create optimal conditions of wetting.

The foregoing observations suggest that an important function of the fat in the cell membrane is to regulate the "wetting" of the cell.

Since glycerine is the carbon compound par excellence from which bacteria synthesize fats, as shown by Larson and Larson,[4]

the necessity for this substance in the nutrition of the tubercle bacillus is obvious.

112 (2072)

The action of potassium cyanide on the chlorophyll mechanism of Nereocystis.

By E. J. LUND and VESTA HOLT (by invitation).

[*From the Puget Sound Marine Biological Laboratory, Friday Harbor, Washington.*]

In connection with studies on the nature of the action of cyanide on cell respiration, it seemed of interest to test the effect which cyanide might have upon the mechanism of photosynthesis.

Strips 1x 10 cm. cut from the frond of the large, Pacific coast kelp Nereocystis were used, and oxygen production was taken as a measure of photosynthesis using Winkler's method for determination of oxygen.

That potassium cyanide (.00008 mol in the experiment given) can produce an apparently complete but reversible inhibition of photosynthesis is shown by the following condensed statement of results from a typical experiment.

TABLE I.

Each number in the table is the average quantity of oxygen produced (+), or consumed (—) in three duplicate determinations. Concentration of KNC in first test, .00008 mol.

Strip placed in.	First test.	Second test. All in light. No KNC.
	O_2 in c.c. thiosulfate.	O_2 in c.c. thiosulfate.
Light	+6.65	+6.89
Light KNC	+ .18	+6.82
Darkness	—1.10	+6.63
Darkness KNC	— .39	+5.80

[4] In this paper the term fat is used as a convenient expression for the ether acetone soluble substances.

Photodynamic sensitization occurs when strips are exposed to light in the presence of higher concentrations (in the experiment cited below .00016 mol.) of cyanide. Injury occurs in proportion to the concentration of cyanide, as shown by table 2, which gives the degree of recovery of the strips, and the mechanism of photosynthesis in terms of capacity to produce oxygen in pure sea water in light, after previous treatment with KNC in light.

TABLE II.

Strips of equal area placed in cyanide solutions (column 1) for nine hours in sunlight. Washed in sea water for eighteen hours and then tested for photosynthesis in pure sea water. Numbers in column 2 give the amounts of oxygen in c.c. thiosulfate produced (+) in a five-hour test.

Strip number.	1. Conc. of KNC in sea water.	2. O₂ in c.c. Thiosulfate.	Condition of strip at end of experiment.
1	Pure sea water	+7.05	Normal color.
2	.00016 mol. KNC	+4.81	Normal color.
3	.00032 mol. KNC	+3.14	Slight loss of color.
4	.00048 mol. KNC	+1.86	More loss of color.
5	.00064 mol. KNC	+ .28	Almost complete loss of color.
6	.0008 mol. KNC	+ .41	Almost complete loss of color.
7	.00112 mol. KNC	+ .22	Almost complete loss of color.
8	.002 mol. KNC	— .31	Complete loss of color.
9	.0032 mol. KNC	— .13	Complete loss of color.

Solutions of cyanide in sea water (about .0006 mol.) which produce permanent injury in light do not produce injury when the strip is placed in darkness, all other conditions remaining the same.

The possible significance and full details of the experiments will be given elsewhere.

SCIENTIFIC PROCEEDINGS

113 (2073)

Studies on fatigue of voluntary muscles.

By LEON ASHER.

[*From the University of Berne, Berne, Switzerland.*]

A new method was worked out to stimulate the nerve of a voluntary muscle of a mammalian animal, this being kept in a physiological condition without impairing circulation, respiration, etc. As no operation was performed the same animal could be used for repeated experiments. Rhythm and strength of tetanic stimuli were controlled.

In the beginning there occurred a slight drop of contraction height, the "initial fatigue." Then a certain level was reached and maintained for several hours. Fatigue did not ensue if the rhythm of tetanic stimuli was every second second, even every second. The ratio of "excitation period" to "pause period" had a marked influence. In isometric contractions non-fatigue was still more definite than in isotonic contractions. Extirpation of the sympathetic nerve supply on one side had no influence.

In the frog, fatigue soon ensued when stimulation was made with tetanic stimuli, but break-shocks of an interval down to every fourth second applied with the new method produced no

fatigue. It appeared at first that stimulation every third second was the critical "interval" but later experience showed that this only holds good for winter frogs. With fresh spring and summer frogs fatigue was avoided in good experiments with stimuli every second second, sometimes even every second. Even when fatigue occurred, if a pause of about twenty minutes was given in a second series of the same stimuli, fatigue did not result. That the observed difference was due primarily to temperature was shown experimentally by placing the frog in a box in which temperature variations ranging between 15 and 35 degrees were obtained. The state of the summer and winter frog could be reproduced in one and the same animal.

Cutting the posterior roots caused fatigue to be delayed on the operated side. Microscopic examinations showed no alterations. Acetylcholin produced the same delay as cutting the posterior roots. An explanation is given, based on the similarity to paralytic secretion after cutting parasympathetic nerves.

Investigations on the action-current of muscles under the new experimental conditions are being carried out with the help of the highly sensitive American-Swiss-Einthoven-Fahr-Stoppani String Galvanometer.

The most recent investigations of the biochemical behavior of the muscles working under the new conditions have shown that formation of lactic acid is about ten times less than with the older methods of studying fatigue.

114 (2074)

Experiments on the metabolism of thymine.

By HARRY J. DEUEL, JR., and LAFAYETTE B. MENDEL.

[*From the Sheffield Laboratory of Physiological Chemistry in Yale University, New Haven, Conn.*]

The recent demonstration[1] of the readiness by which thymine could be oxidized to urea, pyruvic acid and acetol by such mild reagents as $Fe(OH)_3$ and $NaHCO_3$ at room temperature renewed our interest in the metabolism of this pyrimidine in the body.

When 3 gram doses were fed to a small dog (5 kilo), it was possible to isolate approximately one gram of pure thymine from the urine on the following day although none was recovered after administration of the same amount to a large dog (12 kilo). When 3 grams of thymine were given to the same small animal in daily amounts of 0.25 gram over a period of 12 days, it was apparently utilized; for none could be crystallized out of the combined urines of the experimental days, nor was any change in the quantity of non-urea nitrogen noted. The dogs were kept upon constant adequate diets. Experiments on other animals are now in progress.

In every case when thymine was administered, there was an increase in the urea output, suggesting that some of this pyrimidine was metabolized. Attempts to isolate thymine from 150 liters of normal human urine were unsuccessful, thus indicating that under ordinary conditions of diet, thymine does not escape physiological conversion.

Whether thymine ordinarily is destroyed as such in the body or whether still in the nucleoside combination is uncertain; the present experiments suggest that the animal organism is able to convert the pyrimidine to urea when it is uncombined.

The observation of Sweet and Levene[2] that thymine causes diuresis was confirmed when large doses were given; apparently the extent of its diuretic action is dependent upon the amount administered.

1 Johnson, T. B., and Baudisch, O., *Jour. Amer. Chem. Soc.*, 1921, xliii, 2670,

2 Sweet, J. E., and Levene, P. A., *Jour. Exp. Med.*, 1907, ix, 229.

115 (2075)

Certain applications of the Donnan equilibrium to human blood serum.

By DANA W. ATCHLEY, ROBERT F. LOEB, and ETHEL M. BENEDICT
(by invitation).

[*From the Department of Medicine of the College of Physicians and Surgeons, Columbia University, and the Presbyterian Hospital, New York.*]

Some months ago the authors found[1] in comparing some physical and chemical properties of serum and serous effusions that qualitatively certain definite relations obtained, regardless of the nature of the cause of the edema. Some of the relationships observed were: that the potassium content of serum is always higher than that of the effusion, the chlorine content of the serous fluid is higher than that of the serum, while sodium and HCO_3 are apparently equally distributed. It was found, furthermore, that when serum and serous fluid were separated by a collodion membrane and the system was permitted to come into equilibrium these relationships persisted. In this work it was suggested that the relationships existing between serum and serous effusion were the result of a membrane equilibrium and that the quantitative differences were apparently a function of the protein concentration.

In recent experiments we dialyzed human serum against 0.8 per cent. NaCl brought to a P_H of 7.4 by means of $NaHCO_3$ until the serum was practically potassium free. This dialyzed serum protein in various dilutions (made with the NaCl solution) was then dialyzed against 0.8 NaCl at a P_H of 7.4 and at equilibrium, the concentrations of Na and Cl were determined in the serum within the collodion sac and the outside fluid. It was found that [Cl] was greater in the outside fluid than in the serum and the difference was greater the higher the protein concentration in the sac. Apparently the [Na] was greater within the sac containing serum than in the surrounding fluid·

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[1] Robert F. Loeb, Dana W. Atchley and Walter W. Palmer, *Jour. Gen. Physiol.*, 1922, iv, 591.

though the results were not conclusive, owing to considerable error in the method.   When potassium (in amounts similar to those normally present in serum) was added to the outside fluid described in the last experiment and the system was allowed to come to equilibrium, then it was found that—

Concentration K in serum > concentration K in fluid.
Concentration Cl in serum < concentration Cl in fluid.
Concentration Na in serum = concentration Na in fluid.

In other words, when working with pure serum proteins and electrolyte solutions, we are able to reproduce in vitro the conditions existing in the body and the relationships determined are in the nature of the Donnan equilibrium.   Furthermore, as might be predicted from the Donnan equilibrium, the quantitative differences of ion concentrations in the serum and salt solutions are proportionate to the protein concentration of the serum.

116 (2076)

Electrometric acid-base titrations by means of the quinhydrone electrode and its application under physiological conditions.[1]

By VICTOR K. LAMER[2] and T. R. PARSONS.[3]

[*From the Physiological Laboratory, Cambridge University, Cambridge, England.*]

It has recently been shown that an acid solution containing equimolecular proportions of benzoquinone and hydroquinone, a condition which is most easily obtained by dissolving the crystalline addition product, benzoquinhydrone, in the medium under examination, promptly produces a stable and reproducible oxidation-reduction potential on a properly prepared inert electrode, preferably of gold.   In acid solutions the observed po-

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tential has been shown to be a linear function of the P_H and may, therefore, serve as a very simple means of determining hydrogen ion concentrations. For measurements on this system and references to the literature see[4]. Using this method, Biilmann[5] has shown that the potential is stable in a phosphate buffer at P_H 6.81, but unstable in an alkaline borate solution at P_H 9.24, and further that the method possesses the distinct advantage that it may be used where the platinum black hydrogen electrode is inapplicable, e.g., in the presence of certain mild oxidizing agents as 0.1 M nitric acid or unsaturated compounds of the acryhic acid type.

We have made a study of the quinhydrone electrode in order to determine more definitely the range of its applicability under physiological conditions. The validity of the method depends upon maintaining the ratio of the concentrations or more correctly of the activities of the quinone and hydroquinone strictly equal to unity or to some other constant and known value.

The factors which may upset this relationship are: (A) Deviations from the simple oxidation-reduction equation of Peters due to hydroquinone acting as a weak dibasic acid. (B) Changes in the activities of the dissolved quinone or hydroquinone molecules due to the presence of salts. (C) The presence of other oxidizing or reducing substances which react with the quinone substances with measurable velocity.

In order to show the relative importance of these factors we have derived the following general form of the expression for the oxidation-reduction potential (electron pressure) at an inert electrode which will take into account the factors (A) and (B) at varying hydrogen ion and salt concentrations:

$$\text{Observed E.M.F.} = \pi_0 + 0.059 \log A_{H^+} +$$

$$\frac{0.059}{2} \log \left(1 + \frac{K_1}{[H^+]} + \frac{K_1 K_2}{[H^{+2}]}\right) +$$

$$\frac{0.059}{2} \log \frac{A_{quinone}}{A_{hydroquinone}}$$

where π_0 is the normal potential of the quinhydrone system and has the value $+.6990$ volts on the hydrogen electrode

[4] LaMer and Baker, *Jour. Amer. Chem. Soc.*, 1922, xliv, 1954.
[5] *Ann. Chim.*, 9th Ser., 1921, xv. 119.

scale or +.4250 volts when measured against the saturated calomel cell at 25° C.   The temperature coefficient is —.77 millivolts per degree, K_1 and K_2 are the primary and secondary acidic (phenolic) ionization constants of hydroquinone.   The symbol "a" refers to the activity or thermodynamic concentration of the respective substances.

Subject to the restrictions given below this general equation reduces to the simplest form

$$P_H = - \log A_{H^+} = \frac{\pi_0 - observed\ E.M.F.}{0.059}$$

which differs from the usual hydrogen electrode formula only in the magnitude of π_0; namely, by .6990 volts.

From the values for K_1 and K_2, determined by Sheppard[6]; namely, 1.8×10^{-10} and 4×10^{-12}, it can readily be seen that the term involving these constants, which is the correction for factor (A), becomes negligible (less than 0.2 mv.) when the reaction is more acid than P_H 8.0, but that it rapidly increases in magnitude as the solution is made more alkaline.

With regard to factor (B) our experiments have shown that the total salt concentrations present in physiological solutions, except in certain special cases, are seldom sufficient to affect the activities of the quinone substances appreciably, but in any given case the magnitude of this effect may be evaluated and taken into account by determining the activity coefficients from the solubilities of the quinone and of the hydroquinone in pure water and in the salt solution under examination, according to the equations:

activity (a) = activity coefficient (f) × molar concentration (f)

in salt solution $= \dfrac{\text{solubility in salt solution}}{\text{solubility in pure water}}$

In contrast to (A) and (B), factor (C) is important for solutions that are exposed to oxygen owing to the conversion of hydroquinone to quinone which alters the equimolecular ratio between these two substances.   The as yet unpublished results of kinetic experiments by LaMer and Rideal on the mechanism of the autoxidation of hydroquinone have shown

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6 *Trans. Amer. Electrochem. Soc.*, 1921, xxxix, 428.

that the velocity of this reaction varies inversely as (hydrogen ion concentration)$^{3/2}$ and further that it becomes of appreciable magnitude on shaking vigorously in air when the reaction is more alkaline than about P_H 7.8. Apparently hydroquinone will not absorb oxygen until the alkalinity is sufficient to cause phenolic ionization through salt formation.

In order to get more definite information on the stability of the quinhydrone electrode potentials to air over a wide range of P_H, we have carried out careful parallel electrometric titrations at 25° C. of 0.2 molar hydrochloric acid, acetic acid, potassium acid phosphate and borate buffer mixtures with 0.2 molar caustic soda, using in one case the hydrogen electrode and in the other the quinhydrone electrode in the presence of air. We have directed particular attention to the point in the alkaline range at which the quinhydrone electrode first begins to give values that are different from those given by the hydrogen electrode.

The result of these experiments, some of which are represented graphically in the figure, may be summarized as follows:

1. The quinhydrone electrode furnishes an admirably simple and rapid way of determining the end points and titration curves of acids that are stronger than monopotassium phosphate; i.e., having values of K_2 greater than 10^{-7}. The method should be valuable in determining the ionization constants of unsaturated acids.

2. The divergence between the hydrogen electrode and the quinhydrone electrode values is negligible up to P_H 8.0.

3. Since the quinhydrone electrode is a one phase system and therefore attains electrode equilibrium promptly, it can be used for the determination of electrometric end points by continuing the titration curve beyond P_H 8, provided that one works rapidly and avoids undue shaking in air. See boric acid curve for concordance beyond P_H 8.0.

We are extending these investigations to the substituted hydroquinone-quinone systems with the object of choosing appropriate electrode materials for more alkaline ranges and particularly for the determination of the reaction of oxygenated blood.
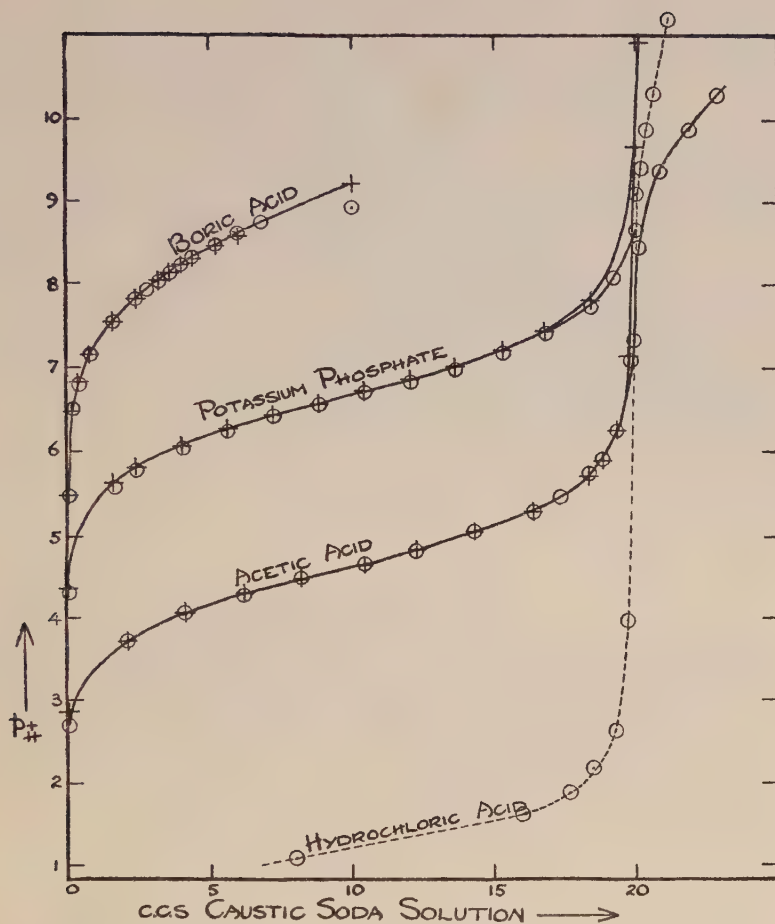
Titration curves obtained with the hydrogen and with the quinhydrone electrodes in the presence of air.

Ordinates—Values of P_H.

Abscissæ—c.c.'s of 0.2 MNaOH added to 20 c.c.'s of the acid component.

+ hydrogen electrode values.

⊙ Quinhydrone electrode values.

117 (2077)

Resistance of pigeons to the lethal action of iletin (insulin) with observed effects on reproduction.

By OSCAR RIDDLE.

[From the Carnegie Station for Experimental Evolution, Cold Spring Harbor, N. Y.]

The pancreatic extract used by us is prepared under the name iletin (insulin, Lilly); it was supplied by the makers and bore the date of preparation. It is prepared by the method of Banting[1] and Macleod[2], one unit of the extract being the amount necessary to reduce the blood sugar of a kilogram rabbit to 0.045 per cent. At this percentage the rabbit often goes into convulsions and death sometimes follows. This unit of iletin or insulin may therefore be considered the lethal dose for a kilogram rabbit.

We are using the pancreatic hormone as a part of a more general study on the relation of the various incretions to reproduction and to sex. Our specific purpose here was to learn whether as a consequence of the hypoglycemia induced by this extract all ovulations would be suppressed in the pigeon by a dosage which should leave the other conditions necessary for reproduction essentially undisturbed. I have earlier reported[3] a marked hypertrophy of the suprarenals at the ovulation period, and in collaboration with Honeywell[4] have shown that parallel with this hypertrophy there regularly occurs in pigeons a marked increase of blood sugar coincident with ovulation. Other unpublished work by Honeywell and myself has made it clear that in each of two very common causes of suppressed ovulation the blood sugar is abnormally low. Such earlier observations have led to the view that a low blood sugar tends to suppress ovulation in birds. This conception is again supported by the results of the present study. It is found that

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1 Banting and Best, *Jour. Lab. and Clin. Med.*, 1922, vii, 464.

2 Banting, Best, Collip, Macleod and Noble, *Amer. Jour. Physiol.*, 1922, lxii, 162.

3 Riddle, PROC. SOC. EXP. BIOL. AND MED., 1922, xix, 122.

4 Honeywell and Riddle, PROC. SOC. EXP. BIOL. AND MED., 1922, xix, 280.

most ovulations are successfully suppressed by subcutaneous injections of quantities of insulin which certainly leave the birds feeding and mating normally, and which do not affect the body weight more adversely (average initial weight = 176g.) than do some other tested tissue extracts which do not suppress ovulation. This depression (12g.) is also not more than the maximum normal seasonal variation.

TABLE I.

Ovulations suppressed in ring doves during one month by subcutaneous injections with one-sixth unit iletin twice daily.

Bird No.	Loss in weight (grams).	Number of ovulations.	
		Expected.	Realized.
1	11	6	0
2	13	6	0
3	4	6	2
4	16	4	0
5	14	4	2
6	14	4	0
7	13	4	0
8	14	4	0
9	15	2	0
10	7	2	0
Total or Average..	12	42	4

Table 1 indicates that not more than one-tenth of the expected number of ovulations are realized under the dosage selected. The indicated number of suppressed ovulations is, however, somewhat too high since only one of the 10 birds used was given blank injections during the control period (upon which the ovulation rate is calculated). Much earlier experience with blank injections and with injections of other tissue extracts shows that this treatment alone appreciably reduces the ovulation rate. It seems certain, however, that the ovulations actually suppressed were equal to more than one-half the number indicated in the table. Only very tame doves were used in this study.

In addition to the data of the table it has been found that this dosage usually does not prevent the ovulation of an egg which is within 48 hours of ovulation at the time of beginning the injections. Again, five birds killed or opened for inspection at three to twenty days after beginning injection showed three cases of degenerating (larger) ova, and two cases in which no ovum has been able to pass into the final stage of

rapid growth.[5] It is thus clear that this quantity of insulin blocks ovulation both by preventing ova from beginning their final period of rapid growth and by sometimes causing them to be resorbed after having entered this stage of growth.

The data (sugar curve for 10 birds) of the accompanying paper[6] show that in the suppressed ovulations of our table 1 the blood sugar was lowered to about .080 per cent.—or one-half its normal value—for a period not longer than four hours twice daily. Depression of the blood sugar to this extent is sufficient to suppress many or most ovulations in ring doves.

In making the necessary tests for the proper dosage for the above purpose it was further learned that the pigeon shows a marked resistance to the lethal action of the extract. This fact

TABLE II.

The resistance of doves and pigeons to large subcutaneous doses of insulin.
Birds not permitted to eat until 48 hours after injection.
(Upper part of table = ring doves; lower = common pigeons).

No. of bird.	Amount of iletin.		Time (hours) no food before injection	Death or survival and remarks.
	Units.	X lethal for rabbit.		
1	5.0	33	21	Died after 5 days; took no food.
2	4.5	30	22	Survived.
3	4.8	30	7	Survived.
4	4.7	30	7	Died* after 28-34 hours.
5	5.0	30	22	Survived.
6	5.0	30	22	Survived.
7	3.2	20	5	Survived.
8	1.3	10	21	Died* after 36-42 hours.
9	1.7	10	0	Survived.
10	1.8	10	0	Survived.
11	1.5	10	0	Survived.
12	1.5	10	0	Survived.
13	1.0	6	0	Survived.
14	1.0	6	0	Survived.
15	1.0	6	0	Survived.
16	10.5	33	0	Died after 8.5 hours.*
17	5.8	18	22	Survived.
18	5.7	18	20	Survived.
19	3.5	10	22	Survived.
20	3.4	10	20	Survived.

*These birds had needle-puncture of heart, or were bled from beak, to obtain sugar samples after injection; this bleeding possibly partly responsible for death.

5 Riddle, *Amer. Jour. Physiol.*, 1916, xli, 387.

is of interest in connection with studies elsewhere now being made upon insulin, since it seems probable that some effects of the hormone scarcely observable in mammals may be successfully studied in the pigeon. Table 2 records our chief observations on this point except for data concerning symptoms and extent of the hypoglycemia induced by heavy dosage.

Certainly some of the symptoms observed in rabbits[2] are sometimes duplicated in the impaired vision and incoördinate movements of the heavily dosed pigeon. These movements strongly resemble those of the ataxic pigeon. Convulsions were observed only in three of the four birds killed by the extract. Unlike the rabbit the bird becomes quiet, apparently very tame (really impaired vision?), and gives little or no evidence of hunger for many hours or days. Though the intestines of most of our heavily dosed birds were made free of food before injection, and though none were permitted to take food earlier than 48 hours after dosage, some birds had to be forcibly fed (impaired vision?) even at the end of 72 or 96 hours. No abscesses have resulted from more than 700 injections.

Two lots or shipments of the extract have been used. The data of table 2 were obtained almost equally from the use of the two preparations. The data of Table 1 are all from the first lot kept in a refrigerator and used when aged one to two months. Its capacity to lower the blood sugar at the end of two months, in one-sixth unit doses, was somewhat less than that of the second lot as tested against the latter within 48 hours after its arrival at the laboratory; the values obtained being .082 per cent. sugar for lot 1, and .062 per cent. sugar for lot 2. These data were obtained as part of the studies reported in the accompanying communication.[6] Other data obtained in connection with that study indicate that though the normal blood sugar of all the kinds of pigeons used is notably higher than that of the rabbit, the value in the pigeon was sometimes reduced to .020 to .040 per cent. without convulsions or death. Bird No. 16 which died after 8.5 hours, gave single unchecked values of .020 per cent. two hours after dosage and .010 per cent. after four hours. Another of the birds which died 28-34 hours after injection gave a value of .025 per cent. after twenty-one hours. Two birds survived though they gave sugar values of .020 and of .030 and .040 per cent.

6 Honeywell and Riddle, PROC. SOC. EXP. BIOL. AND MED., 1923, xx, No. 5.

118 (2078)

The action of iletin (insulin) on the blood sugar of pigeons.

By HANNAH ELIZABETH HONEYWELL and OSCAR RIDDLE.

[From the Department of Physiology, Columbia University, New York City, and the Carnegie Station for Experimental Evolution, Cold Spring Harbor, N. Y.]

The recent preparation of relatively pure and standardized extracts of the sugar reducing principle of the pancreas has been accompanied by measurements of the time and extent of the hypoglycemia resulting from its administration to the rabbit and to man.[1,2] These earlier studies already permit the employment of such extracts with confidence in their power temporarily to reduce the blood sugar level in the higher animals. We present here the result of 120 sugar determinations made after subcutaneous injection of the extract into 35 pigeons of two species and of a third (ataxic) variety. It was usually considered unnecessary to make determinations of the sugar before injection since the birds were handled in groups and the group averages are known from extensive earlier work. These normal averages are: 185 mgms. per 100 c.c. blood (or .185 per cent.) for common pigeons[3]; 161 for ataxics; 150 for ring doves (unpublished data).

In a paper[4] accompanying this communication a pancreatic extract of the standardized type (iletin; or insulin, Lilly) was used continuously over a prolonged period in a test of its capacity to suppress ovulations in the pigeon. Large doses were also used to test the resistance of the bird to the extract. At the close of that study the same group of birds and the same pancreatic extracts were used for the purposes of the present study. One-half of the blood samples were obtained by needle-puncture of the heart and the other half by light bleeding from the upper beak. MacLean's micro-method of sugar determination was

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[1] Banting, Best, Collip, Macleod and Noble, *Amer. Jour. Physiol.,* 1922 lxii, 163.

[2] Sutter and Murlin, PROC. SOC. EXP. BIOL. AND MED., 1922, xx, 68.

[3] Scott and Honeywell, *Amer. Jour. Physiol.,* 1921, lv, 363.

[4] Riddle, PROC. SOC. EXP. BIOL. AND MED., 1923, xx, 5.

used. Very few duplicate determinations were made. Further data concerning the extracts and dosages are given in the accompanying paper.

The curves of Figure 1 show the chief results obtained on ring-doves. In these very tame birds one can obtain samples probably unaffected by emotional glycemia resulting from handling or sampling at one-hour intervals. This is not equally possible with common pigeons and the extent of the fall of the sugar value at one hour after injection was not there measured (Figure 2). But comparison of the curves of the two groups clearly suggests that the maximum effect, of the lighter dosage at least, is reached in less than two hours in the common pigeon as is proved to be the case in the ring dove; probably this limit is attained within one hour.

FIGURE I
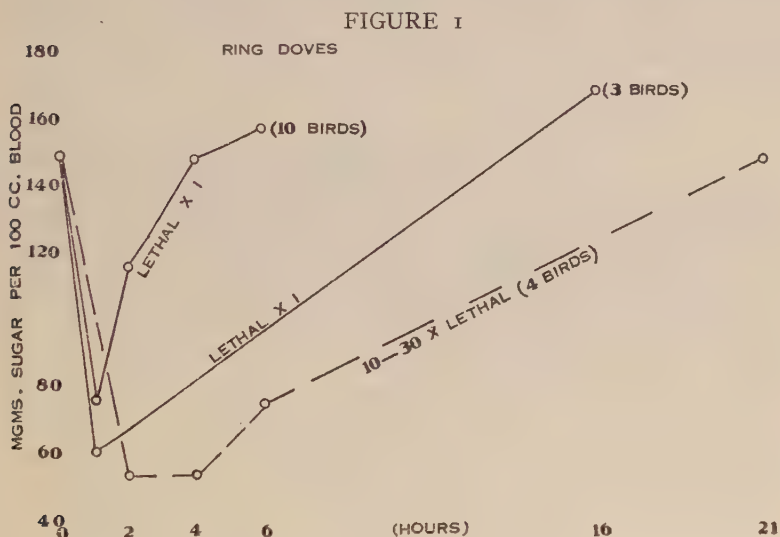
It is notable that in neither of the two groups of common pigeons given light dosage did the sugar return to a normal value at the end of four hours as it clearly did in the ring doves; nor was it at the apparent normal level after 22 hours, but our failure to obtain the normal for these particular birds before injection leaves the point uncertain. It is clear, however, that in the group of 10 ring doves (Figure 1) which at the time of sampling and during preceding weeks was being injected twice daily (9:00 A. M.; 7:00 P. M.) the blood sugar was again

normal after four hours and probably above normal after six hours. Our samples from this group were drawn after the morning injection so that the last previous dosage was thus 14 hours removed. That such previous injection did not affect the sugar values as obtained under this lighter dosage is indicated by the curve itself. This point is further checked by results from the group of 3 birds given only a single injection and whose sugar tested normal or above normal at 16 hours. This group of three ring doves, each injected with one-sixth unit of a very fresh and somewhat more potent extract (Lot 2) than that given to the group of 10 ring doves, gave lower values after one hour (62 mgms.) than did eight birds (82) of the other group which were injected with Lot 1.

Four ring doves given 10 to 30 times the "lethal" dose for the rabbit (Lot 2 used) lowered the sugar value (55) at two hours much more than did the smaller (lethal X 1) dosage (97). Moreover, with the heavy dosage the sugar remained low (55) at four and at six hours. After 21 hours the curve indicates (148) a return to normal value (150); but the figures obtained from the individual birds show that neither of the seven birds for which data for this 19-21 hour period are available was even approximately normal; the figures obtained were: 25, 45, 95, 110, 230, 235, 240 mgms. per 100 c.c. of blood. The four lower values were obtained from birds given the highest dosage —30 times lethal; the three higher values were from birds given 10 (2 cases) or 20 times the lethal dose. The sugar was not determined at the one-hour period in these heavily dosed birds. The low values (20 and 30 mgms.) shown by two birds at the two-hour interval, and the wide difference between the values obtained from low and high dosage at the two-hour interval, together with the demonstrated lower level at one hour than at two, where both were measured strongly suggest that extremely low sugar values obtain at this one-hour period under high dosage. At this particular period, however, the heavily dosed bird shows few or no wide departures from the normal in appearance or behavior. Only one of this group of birds died (after 28-34 hours); its sugar value after 21 hours was 25 mgms. Nearly all of our data from ring doves indicate that heavy insulin dosage causes a very rapid and nearly complete depletion of the blood sugar—to a point not higher than one-sixth its normal value; that this is usually followed by a some-

FIGURE 2
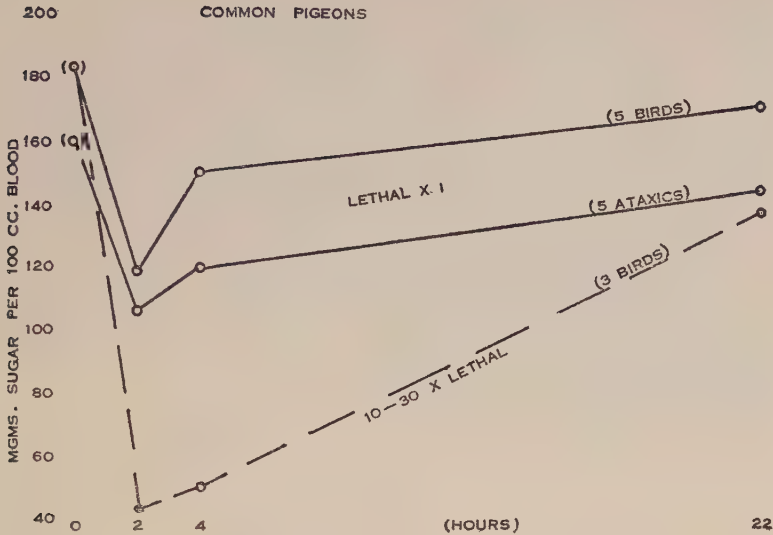
what slower and less complete rise; and that the short period of nearly complete absence of sugar is a period of little danger to the life of the bird.

The upper and lower curves of Figure 2 give for normal common pigeons the sugar measurements obtained from low and high dosage respectively. The much lower values from and the prolonged effect of the heavy dosage are apparent; they are also quite similar to the differences obtained with ring doves. The five ataxic pigeons figured on the middle curve were earlier known to have lower sugar values than normal pigeons. They maintained this lower level when under equivalent dosage. All these birds had a body weight of about one-third kilogram and received therefore in the "lethal X 1" dosage about one-third unit of iletin. In the "10 to 30 X lethal" dosage they received 3 to 10.5 units of iletin. Besides having a lower body weight which results in a saving of injected material the ring doves offer several advantages over the more commonly used pigeon for laboratory studies involving the determination of blood sugar.

The blood sugar was measured in a few instances at intervals longer than 24 hours after injections. For five of these birds the sugar was not measured soon after injection and are there-

fore not represented on the curves noted above. Three ring doves at 34, 35 and 36 days after injection with 1 unit (6 X lethal) gave normal average values of 143, 145 and 155 for these three days; one dove given 4.5 units showed a normal value (155) at 48 hours, but less (110) at 74 hours. One common pigeon (18 X lethal) gave values of 165 after 48 hours and of only 70 after 74 hours; this bird showed only 15 mgms. sugar after two hours and survived the treatment. Another common pigeon given 3.4 units (10 X lethal) gave a normal value (185) at 32 hours and less than one-half normal (70) at 58 hours. Some of these heavily dosed birds whose sugars were low at advanced periods had probably taken no food during a considerable preceding interval..

The fact that many of the ring doves and common pigeons given heavy dosage of insulin were not permitted to take food during about 20 hours preceding dosage, and that sugar samples representing values recorded in the curves were taken as much as 22 hours after injection, raises a question as to the bearing of this circumstance upon the sugar values obtained. Concerning this point it is noted that earlier studies[5] have indicated that inanition during 48 hours is practically without effect on the normal blood sugar of the pigeon. Only 7 of the 30 birds included in the curves presented (10-30 x lethal) were in any way restricted in their feeding either before or after injection of the extracts.

119 (2079)

Experimental production of streptococcus endocarditis with glomerular nephritis.

By RALPH A. KINSELLA and C. C. SHERBURNE.

[From the Medical Clinic, Barnes Hospital, Washington University School of Medicine, St. Louis, Mo.]

In human patients subacute streptococcus endocarditis is a fatal disease. No authentic report of a recovery has even been published. The mechanism of production of this disease in

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5 Honeywell, *Amer. Jour. of Physiol.*, 1921, lviii, 152.

human beings displays two constant factors—injury to the valve and later infection. The injury is usually represented by either congenital valvular heart disease or rheumatic valvular heart disease. The later implantation of green streptococcus on this injury usually takes place through the medium of an infection of the middle ear or throat or some other locality where green streptococci normally breed. In experimental work Rosenbach recognized these two factors in 1878 and reproduced infection in the heart valve after puncture of the valve. Although a beginning was made so long ago, and more perfect instruments have been devised for injuring the valve, no attempt has been made to reproduce the disease completely and glomerular nephritis of the type which characterizes the disease in human beings has never heretofore been reproduced. All clinical efforts to obtain a cure of the disease have failed. It seems, therefore, that we must have the disease reproduced in animals and then thoroughly study its features if we are to expect a cure.

This work consists in injuring the aortic valve by inserting an appropriate instrument into the left carotid and then, after recovery, the animal is infected by intravenous inoculation of green streptococcus. The inoculated bacteria become implanted at the site of the valve injury and there set up a bacterial vegetation identical with that of human patients. Dogs were used in these experiments. Dogs living 12, 13 and 14 days failed to show any kidney lesion of the glomerular type although large infractions were common. A longer survival of the animal seemed essential for producing the kidney picture. The gross specimens exhibited here and the microscopic specimen showing the glomerular lesion are from a dog which lived 17 days. The glomerular lesion consists of partial thrombosis of the tuft with hyaline degeneration and with hemorrhage and infiltration with polymorph. leucocytes. One of the most important results of this work is that we have reproduced a bacterial infectious disease which is of sufficient duration to permit thorough study of many of the unknown factors of infection and immunity.

120 (2080)

A criticism and modification of the MacLean blood sugar method.

By A. BAIRD HASTINGS and ALEITA HOPPING.

[*From the Department of Physiology, Columbia University, New York City.*]

The titrimetric method for the determination of blood sugar proposed by Maclean[1] in 1919 has been used with success in a number of researches both abroad and in this country. Its simplicity, accuracy and the small amount of blood required recommend it to those who prefer titrimetric to colorimetric quantitative determinations. Briefly, the principle of the MacLean method is:

1. Removal of the proteins by heating oxalated blood with acidified sodium sulphate, adding colloidal iron, and filtering.

2. Boiling, under standardized conditions, an aliquot part of the filtrate with a known amount of an alkaline copper solution containing potassium iodide and potassium iodate.

3. Titration of the iodine liberated upon acidification with standard thiosulphate.

Since, under the conditions defined by MacLean, the cuprous salt reduced by the sugar is oxidized by the iodine liberated, the excess iodine titrated with thiosulphate is inversely proportional to the amount of reduction. Empirical factors can, therefore, be determined converting the volume of thiosulphate used into concentration of glucose.

It was found, however, that by MacLean's method the determinations of sugar in freshly drawn blood to which no anticoagulant had been added gave values distinctly higher than those determined on the same blood which had been oxalated, (Table I).

TABLE I.

Sugar determinations of blood to which $K_2C_2O_4$ had been added and had not been added.

Method.	Dog.	Concentration of sugar in mgs. per 100 c.c. of blood.					
		$K_2C_2O_4$ present.			$K_2C_2O_4$ not present.		
		a	b	Average	a	b	Average
Original........	A	105	100	103	89	88	89
MacLean........	B	91	92	92	55	55	55
Modified........	C	90	85	83	90	85	88
MacLean........	D	93	96	95	93	93	93

[1] MacLean, H., *Biochem. J.*, 1919, xiii, 135.

This fact led to a comparison of sugar determinations made on pure glucose solutions with and without the addition of oxalate, with and without the use of colloidal iron. The amount of oxalate required to prevent coagulation was about 2 mg. per c.c. It was found that the presence of the oxalate resulted in too low sugar values, *i.e.*, too much thiosulphate was used in the titration, only in case colloidal iron was used. Experiments in which the amount of colloidal iron was progressively increased from 0 to 3 c.c. showed that increasing amounts of thiosulphate were required for titration. Direct determinations of the iron in the filtrate indicated that the reaction involved was between the thiosulphate and the ferric salts. It seemed, therefore, that the choice of colloidal iron as a protein precipitant was an unfortunate one; although, under the carefully standardized conditions described by MacLean, it led to no inaccuracy of results, providing oxalate was not used as an anticoagulant.

By using phosphotungstic acid as our protein precipitant we were able to obtain results which agreed whether the blood was oxalated or not, and which were comparable with those obtained by the Folin method.

The procedure now adopted for the precipitation of the blood proteins is as follows:

1 c.c. of oxalated blood is added to 26 c.c. of distilled water in an Erlenmeyer flask or 50 c.c. centrifuge tube. Five minutes are allowed for laking the blood. 2 c.c. of a 10 per cent. solution of phosphotungstic acid and 1 c.c. of 1 per cent. acetic acid are added. The flask or tube is then vigorously shaken. In the case of pigeon's blood, where precipitation is difficult, it is necessary to gently heat the mixture after the addition of phosphotungstic acid to complete the action. The protein precipitate may then be separated by filtration or preferably centrifugation. 20 c.c. of the water-clear filtrate are transferred to an Erlenmeyer flask, and the determination continued as recommended by MacLean.

The elimination of sodium sulphate from the solution results in the reduction taking place at a lower temperature. This necessitates the construction of a new table to take the place of the one published by MacLean for the conversion of c.c. of thiosulphate into mgs. of glucose per 100 c.c. of blood. These equivalents are given in Table II.

The method as now modified has been successfully applied to human, dog, ox, rabbit, guinea pig and pigeon blood.

TABLE II.

Table for the conversion of c.c. of N/100 $Na_2S_2O_3$ into mg. of glucose per c.c.

0.01N $Na_2S_2O_3$ c.c.	Glucose mg. per c.c.	0.01N $Na_2S_2O_3$ c.c	Glucose mg. per c.c.
0.50	0.25	4.0	1.19
1.0	0.39	4.5	1.33
1.5	0.52	5.0	1.46
2.0	0.66	5.5	1.59
2.5	0.79	6.0	1.73
3.0	0.92	6.5	1.76
3.5	1.06	7.0	2.00

121 (2081)

On the nature of the rhythmic contractions in the stomach and intestine.

By ALBERT KUNTZ and J. EARL THOMAS.

[From the Departments of Anatomy and Physiology, St. Louis University School of Medicine, St. Louis, Mo.]

Rhythmic contractions in the stomach and intestine persist following the administration of nicotin in doses sufficient to prevent conduction through synapses. Attempts to account for these contractions solely as responses to nervous impulses have resulted in confusion. Certain experimental data recorded by Magnus ('05),[1] Gunn and Underhill ('14),[2] and Alvarez and Mahoney ('22)[3] indicate clearly that excised pieces of the intestinal musculature may execute rhythmic contractions in the absence of nervous influences. The present paper embodies a preliminary statement of the results of a further investigation, through the use of nicotin in massive doses, of the rhythmic

[1] Mangus, R., Arch. f. d. gesammt. Physiol., 1905, cviii, 1.

[2] Gunn, J. A. and Underhill, S. W. F., Quart. Jour. Exp. Physiol., 1914, viii, 275.

[3] Alvarez, W. C. and Mahoney, L. J., Amer. Jour. Physiol., 1922, lix, 421.

contractions both in the stomach and intestine in intact animals (dog).

Nicotin hydrochloride was administered intravenously in successive gradually increasing doses. Artificial respiration was employed as early as necessary and throughout the rest of the experiment. Segmental contractions in the small intestine, peristalsis in the stomach, rhythmic contractions of the pyloric sphincter, and antiperistalsis in the large intestine continued after the administration of sufficient nicotin to abolish all responses either to electrical stimulation of postganglionic sympathetic fibers or the administration of adrenalin in relatively large doses (0.01 to 0.025 mg. per kg. of body weight). At this time the administration of adrenalin brought about no change in blood pressure. It was found necessary, in order to secure these results, to administer approximately three grams of nicotin hydrochloride per kilogram of body weight. If now sufficient time was allowed without the administration of more nicotin the conducting mechanism recovered to such an extent that responses were again elicited by electrical stimulation of postganglionic sympathetic fibers or the administration of adrenalin in moderate doses.

The failure of relatively large doses of adrenalin, following the administration of nicotin in the quantities stated above, to bring about inhibition of the rhythmic contractions in the stomach and intestine indicates at least a very great reduction in the irritability of the myoneural junction. The failure of electrical stimulation of postganglionic sympathetic fibers to bring about any modification of these contractions indicates paralysis of the nerve fibers or the myoneural junction or both. Therefore, we may assume that the rhythmic contractions in the stomach, pyloric sphincter, and the small and large intestine which continue when this stage in the nicotin paralysis is reached do so in the absence of nervous influences. Consequently, the capacity to execute rhythmic contractions is inherent in the gastro-intestinal musculature. Furthermore, the rhythmic contractions above indicated in the several parts of the gastro-intestinal canal probably belong to the same category of functional motility.

The results of these experiments do not demonstrate the absence of nervous control, under normal conditions, of the rythmic contractions in any part of the gastro-intestinal canal. The tracings recorded throughout the progress of any successful ex-

periment in our series indicate progressive changes in the configuration and amplitude of the contractions as the nervous paralysis brought about by the nicotin advances. These tracings will be analyzed in detail in a later paper. A few of the more striking changes may be stated as follows: Both in the stomach and intestine, before the administration of nicotin, the rhythmic contractions vary greatly in amplitude and are commonly superimposed on large tone changes. As the nicotin paralysis advances the tone changes subside and the amplitude of the contractions, while becoming more uniform, diminishes greatly until a stage is reached at which apparently certain inhibitory influences are removed and the amplitude of the contractions increases suddenly and markedly. After this the rhythmic contractions continue with a high degree of regularity. As the nicotin paralysis is carried still farther the amplitude of the contractions gradually decreases but is still relatively large when electrical stimulation of postganglionic sympathetic fibers is no longer effective. If the administration of nicotin is discontinued at this point the rhythmic contractions continue without marked changes in amplitude.

We do not maintain that the tracings recorded before the administration of nicotin in these experiments represent the exact configuration of the contractions going on in the stomach and intestine under normal conditions. Nevertheless, we believe that the changes which occur in the tracings during the progress of the nicotin paralysis represent actual changes in the muscular activity which are due largely to the elimination of nervous influences. Doubtless, rhythmic contractions in the gastro-intestinal canal are normally subject, in a large degree, to nervous control.

122 (2082)

Narcosis and temperature.

By M. E. COLLETT.

[*From the University of Buffalo, Buffalo, New York.*]

If the lipoid theory of narcosis is true, we should expect to find the narcotic power of a substance and its oil water partition coefficient similarly affected by changes in temperature. Thus, benzamide and salicylamide should increase in efficiency as the temperature falls, while chloral hydrate should increase as the temperature rises. The experiments here reported were made in 1921-22 at Woods Hole (M. B. L.) and at the University of Buffalo in order to test the question, since recent literature has reported rather conflicting evidence.

My experiments showed that in some cases, (swimming movements of *Loligo* and *Gonionemus,* and heart-beat of *Perophora*) the rule holds at least over the range of concentrations tested. In other instances (swimming of toad and frog tadpoles, *Bdelloura* and *Arbacia* gastrulæ) the rule holds for chloral hydrate at all concentrations, but for benzamide and salicylamide only at low concentrations. In still other cases (cilia of *Nereis* gastrulæ, gill cilia of *Venus* and *Pecten,* tentacles of *Metridium,* chromatophores of *Loligo*) the rule holds only for chloral hydrate; benzamide and salicylamide, instead of increasing in efficiency as temperature falls, regularly decrease at all concentrations tested. With a few tissues (hearts of *Nereis* and *Limulus,* chromatophores of *Fundulus,* cilia of *Arenicola* larvæ) a fall in temperature increases the efficiency of chloral hydrate as well as of salicylamide and benzamide; but as these tissues are easily narcotized by cold alone, the results are of doubtful significance.

From these experiments it would seem that the lipoid theory of narcosis holds true, at least for many tissues, provided that dilute solutions are used. With stronger solutions other factors than lipoid solubility may enter in and so cloud the results.

123 (2083)

On the intravascular development of erythrocytes in the bone marrow of the adult pigeon.

By CHARLES A. DOAN (by invitation).

[*From the Department of Anatomy, Johns Hopkins University, Baltimore, Maryland.*]

The observations of Maximow[1] and Danchakoff[2] in fixed tissue, and of Sabin[3] in the living blastoderm, have demonstrated that the red blood cells differentiate intravascularly in the embryo. Maximow and others have contended that the adult bone marrow differs from the embryological vascular areas in the method of producing red cells. They believe that, in adult marrow, the erythrocytes develops in extravascular clumps, the mature cells later making their way into the bloodstream. This has been so generally accepted that most recent workers have concentrated on attempting to determine the mechanism whereby the adult erythrocytes obtain entrance to the circulation.

In studies on the vascular pattern of the pigeon's marrow with an hypolasia induced experimentally by starvation, an extensive system of intersinusoidal, collapsed capillaries lined by an embryological type of endothelium was observed for the first time.[4] These capillaries, evidently, are not normally patent to the circulating blood as are the transition capillaries which connect arterioles and venous sinuses. The cellular elements in the hypoplastic marrow are reduced to three types: fat cells, reticular cells, and endothelial cells; and the depleted cellular structure of the marrow is replaced by an increase of fat deposit.

The hypoplastic marrow of the starved animal recovers rapidly upon the resumption of an ordinary diet thus providing a simple physiological method of experimental control without

1 Maximow, A., *Arch. f. mikr. Anat.*, 1909, lxxiii.

2 Danchakoff, W., *Arch. f. mikr. Anat.*, 1909, lxxiii, 117.

3 Sabin, F. R., *Contributions to Embryology*, 1920, ix, 213, Carnegie Inst. of Washington, Publ. 272.

4 Doan, C. A., *Contributions to Embryology*, 1922, xiv, 27, Carnegie Inst. of Washington, Publ. 277.

the introduction of any complicating factors. When feeding is resumed and observations are made at varying intervals thereafter, it is possible to secure a series of marrows of various degrees of complexity, as normal cellularity is approached. An analysis of each stage, from the simple depleted to the normally cellular state, enables a clarity of interpretation and understanding of the normal process of blood-cell formation and development hitherto unknown. The phenomena revealed by this series have proved highly suggestive.

Forty-eight hours after the resumption of feeding in an animal with a previously induced hypoplasia, a most remarkable change in the appearance of the marrow is to be seen. The excessive deposit of fat has very largely disappeared. This is evidenced by a marked stellate appearance of the shrinking fat cells, with the fat therein divided into many various sized globules, in contrast to the hypoplastic state where it was deposited as one homogeneous mass in the large fat cells. In this stage the fat cells simulate clasmatocytes in appearance, and are most numerous along the outsides of the blood vessels. There is in addition to this a most striking proliferation of endothelium. Because of the prominence of the strands of endothelial cells, their distribution is very readily appreciated. There remains no doubt in this stage that the inter-sinusoidal channels which in injected hypoplastic marrow are seen surrounding the fat cells, are true endothelial capillaries, for the proliferated endothelium follows precisely and accurately their pericellular, intersinusoidal outline. The picture described is not that seen in a few isolated areas, but it is a transformation in which the entire marrow substance participates. Here and there are to be seen a few young red blood cells, all intravascular, and in contact with the swollen endothelial cells of a collapsed capillary. In the extravascular parenchymal spaces there is to be seen an occasional so-called "reticular cell' in the process of mitotic division. The "reticular cells" of the hypoplastic state are large irregular pentagonal or hexagonal cells with faintly staining eosinophilic cytoplasm, and round vesicular nuclei; simulating in appearance primitive mesenchyme.

In the marrows analysed after seventy-eight hours of stimulation most of the fat deposit has entirely disappeared, and only a few vacuolated cells remain. There is still more extensive proliferation of the capillary endothelium than at forty-eight

hours and a veritable honey-comb design is outlined by the vessels, a small number only of which seem to be completely open to the circulation.   There are very many developing red blood-cells, all within strands of endothelium.   In some places it appears almost as though the endothelium were being actually replaced by a strand of developing red blood-cells, though the regularity and continuity of outline is nowhere broken.   There are numerous small groups of from five to eight young granulocytes in various stages of development, all located extravascularly in the parenchyma.   Succeeding stages increase the complexity of the picture.   However, insofar as we have been able to observe, the red blood-cells have appeared only intravascularly, and the white blood-cells extravascularly.   No analyses of hyperplastic marrows have as yet been attempted.

124 (2084)

On the intravascular development of erythrocytes in the bone marrow of the adult rabbit.

By R. S. CUNNINGHAM and C. A. DOAN.

[*From the Department of Anatomy, Johns Hopkins University, Baltimore, Maryland.*]

Bone marrow has been one of the most difficult tissues to understand because it has proved so hard to reduce it to a sufficiently simple state for analysis.   The older methods, consisting chiefly in classification of cell types, have led to the almost universal acceptance of the monophyletic theory, and in general to the conclusion that the developing red blood cells are formed in parenchymal spaces outside the vascular system, hence differing from the manner of development found in the embryo. Since it has been accepted that the red cells develop extravascularly, it has been obviously necessary to determine their mode of entry into the circulation.   The two principal explanations offered have been: (1) That the endothelial lining of the vascular bed was incomplete, as in the spleen, and consequently the young cells could be forced through these openings; and

(2) that the growing clumps of red cells cause erosion of the endothelial lining by pressure, and hence obtain entrance to the vascular bed. Drinker, Drinker and Lund[1] were able to demonstrate that the endothelial lining in normal marrow is quite complete, but that in hyperplastic marrows this is probably not the case. They found that the injection mass was not so evenly outlined by the walls of the sinusoids in the hyperplastic as in the normal marrows. This difference they explained as due to young red cells which had eroded the endothelial wall by pressure, while, at the same time, these cells were so closely packed together that they prevented the injection mass from extravasating freely into the parenchyma.

Our concept of the normal structure of the vertebrate bone marrow has been considerably modified by the demonstration by one of us[2] of a very elaborate capillary bed in the marrow of the adult pigeon. These capillaries were first demonstrated by injection, but it was found that they could only be satisfactorily injected after the marrow had been reduced to a very simple hypoplastic state by starvation. The demonstration of a similar vascular pattern in the mammalian marrow has been very difficult, and yet such a study is essential since, if these capillaries occur in mammals as well as birds, it will be of the greatest importance to hematology. The vast number of investigations of the bone marrow which have been carried out on anemias produced by various toxic agents, as *e.g.,* Thorium-X and other radioactive substances, have caused too much injury to the marrow to allow analysis of the normal structure. Starvation has so far proved relatively ineffective in the mammal and no other satisfactory method has yet been found for reducing the marrow to the hypoplastic state which has been obtained in the pigeon.

We have been able, however, by a very simple procedure, to obtain a marrow in the rabbit in which the myelocytic cells are very largely removed and only the developing reds retained; in such a marrow it has been immediately possible to see that there are large numbers of young red cells definitely within capillaries which are not part of the ordinary blood filled sinuses

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[1] Drinker, Drinker, and Lund, *Amer. Journ. Physiol.,* 1922, lxii, 1.

[2] Doan, *Contributions to Embryology,* 1922 xiv, 29, Carnegie Inst. of Washington, Publication No. 277.

and which are running from sinus to sinus as shown by Doan in the pigeon. With endothelium, as delicate as this is, it is obviously impossible in sections to always determine the exact location of the red blood cells, but a large proportion of them appear in rows extending from sinus to sinus, with here and there parallel, investing, endothelial nuclei. The histological picture obtained is strikingly similar to that described for the pigeon's marrow during recovery from starvation,[3] and in view of such a similarity our conclusion that most of the red cells arise intravascularly seems wholly justified.

The method that we have used for this purpose has been the administration of large doses of dead typhoid bacilli intravenously. Our doses have been increasingly large, usually beginning with ½ of a 24 hour agar slant, planted in a standard manner, and increasing to 1, 2 and 3 cultures to the dose. Very large outpourings of leucocytes have immediately occurred and after 3 doses of this character the bone marrow has been depleted to the maximum of its myelocytes and has not begun to form new white cells to any appreciable extent.

The conclusion that the red cells arise by proliferation of endothelial cells which remain in the bone marrow in a relatively undifferentiated state seems justified from the observations of Doan on the pigeon and those reported here for the rabbit. That large clumps of developing red cells do occur in the marrow has been observed many times, and though an endothelial margin can not always be demonstrated, this may be either the difficulty of demonstrating so delicate a structure in sections, or else an overgrowth of the young cells into a blood island. Either conclusion obviously explains the method of entry of the adult erythrocytes into the circulation through the original openings of the capillaries into the sinusoids. It has been determined that endothelium gives rise to the erythrocytes of the embryo in a large number of species; and suggestive evidence is presented here for the mammal, and elsewhere for the pigeon, that a similar relationship exists in the adult marrow. This seems to us to indicate an additional and most important physiological significance for endothelium in the adult vertebrate.

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3 Doan, Proc. Soc. Exp. Biol. and Med., 1923, xx, 5.

125 (2085)

A phyto-pharmacological study of a menotoxin or menstrual toxin.

By DAVID I. MACHT and DOROTHY LUBIN.

[*From the Pharmacological Laboratory, Johns Hopkins University, Baltimore, Maryland.*]

Macht and Livingston have already shown in connection with a study of cocain and its derivatives that various drugs affect animal and cell protoplasm very differently. Thus it was shown that while cocain is very toxic for animal tissues, it is comparatively little toxic for plant protoplasm. On the other hand, sodium benzoate which is practically non-toxic for animal tissues was found to be extremely toxic for the root of *lupinus albus*. These observations suggest the idea that plant cells may be much more sensitive to some animal toxins than animal cells or tissues might be. This idea was a starting point for the present investigation. Shick has recently revived or called attention to the ancient popular belief as to the contaminating or deleterious effects of the touch of women at the time of menstruation. He performed a few experimnts on cut flowers seemingly corroborating this idea. The present authors decided to investigate this whole question in a more scientific and accurate way by the use of whole living plant organisms and not cut flowers, inasmuch as the latter method is unreliable for obvious reasons. The procedure was very much the same as in the study of Macht and Livingston on cocain and its derivatives.

Seedlings of *lupinus albus* were grown in a perfect nutrient medium (Shive solution) and the rate of growth of the single straight well defined root was measured to within one-half of a millimeter. Similar seedlings were grown simultaneously and under exactly the same conditions in Shive solution containing a definite amount, usually 1 per cent. of normal blood serum, and on other occasions exactly the same kind of experiments were performed with solutions containing blood serum obtained from menstruating individuals. Whenever possible normal and pathological blood was obtained from the same subjects. The effect of normal serum on the growth of seedlings as compared

with normal Shive solution was determined and in a similar way the effect of menstrual serum on the growth of seedlings was ascertained. It was found that while the average growth of the seedlings in a 1 per cent. solution of normal blood serum in Shive solution gave about 75 per cent. of growth as compared with the normal Shive, a similar solution of menstrual serum affected the seedlings in a much more toxic way. This toxicity expressed itself in two ways, in the first place the absolute growth in the length of the roots was much less, on an average about 50 per cent., as compared with the normal seedlings. In the second place, the rootlets no longer grew in a perfectly straight direction, but were curled and distorted in various ways. Numerous experiments repeatedly performed in the above way with numerous samples of sera from normal and menstruating subjects as compared with normal ones. The greatest toxicity was usually found at the beginning of menstruation and was demonstrable even during the premenstrual period of a day before the actual onset of the catamenial flow, growth in such cases being as low as 30 per cent. of the normal.

Similar experiments were made with samples of saliva obtained from normal and menstruating subjects and difference in toxicity was conclusively demonstrated in the case of that secretion also. It was also found that what we may call the "menotoxin" was present likewise in the red blood cells and some very striking experiments revealed the presence of the same menotoxin in the skin secretion of menstruating individuals. Experiments are in progress with a view of ascertaining more intimately the chemical nature of the menotoxin. Various glandular extracts, such as ovarian, corpus luteum, thyroid suprarenal, pituitary, etc., have been examined and more definitely known chemical compounds have also been studied. Fuller data will be published in due time.

126 (2086)

Intravenous injection of hemoglobin in the treatment of anemia.

By ALICE R. BERNHEIM (by invitation).

[*From the First Medical (Cornell) Division, New York Hospital,
New York City.*]

Brugsch and Yoshimoto[1], and Whipple and Hooper[2] have shown that hemoglobin, when introduced intravenously into bile fistula dogs, is converted a short time after injection into bile pigment. Whipple and Hooper have also shown that bile pigment circulation does not exist, but that bile pigments are excretions.

On the other hand, McMaster and Haessler,[3] of the Rockefeller Institute, using rabbits, have demonstrated that hemoglobin intravenously injected cures anemia.

With these observations in mind, hemoglobin injections were given to five patients in the New York Hospital (First Medical Division). Altogether eleven injections have been given. The hemoglobin was prepared from fresh human blood. There were two cases of pernicious anemia; two of secondary anemia, and one of myelogenous leucemia. The results are encouraging, and the work is being continued.

[1] Brugsch and Yoshimoto, *Zeitsch. f. Exper. Path. u. Therap.*, 1910-11, viii, 639.

[2] Whipple and Hooper, *Am. Jour. Physiol.*, 1917, xliii, No. 2.

[3] McMaster and Haessler, *Jour. Exp. Med.*, 1921, xxxiv, 579.

127 (2087)

An improved procedure for metabolism experiments.

By GEORGE R. COWGILL.

[*From the Sheffield Laboratory of Physiological Chemistry in Yale University, New Haven, Connecticut.*]

The literature concerning metabolism contains many accounts of failures of experiments due to refusal of the animals—usually dogs—to eat the diets offered.[1] Investigations into the physiology of vitamin-B as carried out at this laboratory have shown this accessory food substance to be a factor essential for the maintenance of the appetite. This fact is of peculiar interest to students of metabolism.

We are now able to give quantitative expression to this fact in terms of the source of vitamin-B which we have tested, and have utilized this relationship to make dogs eat during the periods of metabolism experiments.

A dog receiving a synthetic diet adequate in all respects except vitamin-B, and containing only enough calories to maintain a fairly constant body weight, requires for the maintenance of appetite approximately 50 milligrams of "Yeast Vitamine (Harris) Powder" per kilogram of body weight per day. Our procedure in metabolism experiments has been to weigh out the daily doses, place them in gelatin capsules, and to give one capsule to the animal each day.

This method has been tested by workers at our laboratory with entirely satisfactory results. Dogs have been maintained on purely synthetic diets with remarkably constant daily output of nitrogen over unusually long periods.

Experiments designed to determine the minimum daily dose of a wheat embryo preparation containing vitamin-B are now in progress.

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[1] Many might be cited. More striking instances are: Forster, J., *Zeitsch. f. Biol.*, 1873, ix, 297; Steinitz, F., *Pflüger's Archiv.*, 1898, lxxii, 75; Abderhalden, E., and Oppler, B., *Zeitschr. f. Physiol. Chem.*, 1907, li, 226.

128 (2088)

The value of gelatine and gelatine preparations in the diet of man.

By PHILIP B. HAWK.

[*From the Laboratory of Physiological Chemistry, Jefferson Medical College, Philadelphia, Pa.*]

Four albino rats were given a diet of dried bread, 80.5 per cent.; butter, 15.0; salt, 2.0; and yeast, 2.5. Four others were given the same diet except ten parts of bread were replaced by ten of granulated gelatine. At the end of seventeen weeks the first group attained an average weight of 113 grams, and the second group an average weight of 194 grams. The gelatine thus supplemented the cereal diet with respect to protein, probably due to its high content of lysine in which cereals are low.

One hundred c.c. portions of plain milk and of milk containing one per cent. of gelatine were given to four normal men on successive days. Gastric digestion was followed by the fractional method. Finer and softer curds were formed with gelatine-milk than with plain milk. The hydrochloric acid was more rapidly and completely combined. The digestion time was shortened.

Eight children from three to eight months of age and suffering from indigestion or malnutrition, some with large curds in vomitus and stools, were placed on gelatine-milk for from six weeks to four months. A decided improvement in nutrition was noted in all cases and no untoward effects were observed.

Fifty persons suffering from tuberculosis were given gelatine in addition to their regular egg-milk diet. Thirty-five showed definite improvement. In most of the other cases intestinal ulcerations and other complications existed. The effects noted were probably due largely to the better utilization of milk.

The digestibility in the human stomach of a number of the more common gelatine preparations was studied by the fractional method. Four hundred c.c. of 1.5 per cent. gelatine left the stomach in one hour. Fruit juice preparations left the stomach almost as rapidly. Those containing cream remained a little longer. Because of the extreme ease with which gelatine preparations are digested and their appetizing character, they

are especially suitable for the diet of convalescents. Thus orange gelatine was more easily handled by the stomach than the commonly used orange albumin. The preparations containing milk and eggs serve as vehicles and make it possible to increase in the diet the amount of these foods for which some persons (especially children) may have a distaste.

In ten individuals showing hypo- or hyper-acidity, gelatine was also readily digested. It should prove of value in stomach disorders because of the slight burden it places on the digestive functions, its acid-combining power, and low degree of acid stimulation.

Gelatine preparations made from fresh orange, lemon and strawberry juice with the degree of heating commonly employed in the household showed essentially the pronounced antiscorbutic action of the fresh juices themselves. Scurvy in guinea pigs was readily overcome by small amounts of such preparations.

A study was made of the indican and phenol elimination of a normal man on a diet, the protein portion of which was supplied by gelatine. Knox gelatine was used in this as in the other tests, 72 grams per day being given for five days. In another period a meat diet was given. The indican output fell from 8.25 mg. on the last day of the meat period to 0.70 mg. on the last day of the gelatine period. Total phenols fell from 506 to 193 mg. The period averages were 474 and 331 mg. respectively. The decreases in indican and phenols were probably due to the low content of gelatine in tryptophane and tyrosine.

129 (2089)

The cultivation of the organisms of rocky mountain spotted fever and typhus in tissue cultures.

By S. B. WOLBACH, HENRY PINKERTON, and
MONROE J. SCHLESINGER.*

[From the Departments of Pathology and Bacteriology, Harvard Medical School, Boston, Massachusetts.]

In these experiments tissues from infected adult guinea pigs were grown in plasma obtained from normal guinea pigs. With

Rocky Mountain spotted fever the bits of tissue were taken from the tunica of the testes. With typhus the cerebral cortex was used. The plasma was obtained from adult normal guinea pigs by centrifuging chilled blood collected in paraffined tubes.

Our results with spotted fever prove that the virus survives and multiplies in such cultures. Evidence of survival of the virus was obtained by causing the disease in guinea pigs by injecting the cultures intraperitoneally. Although the spotted fever reaction is very characteristic, the results were controlled by histological studies or by subsequent immunity tests.

Evidence of multiplication of the virus was obtained by demonstration of the minute paired micro-organism of the disease (Dermacentroxenusrickettsi) in increasing numbers in first generation cultures up to about the fourteenth day. The micro-organisms are always intracellular, in large amœboid phagocytic cells of endothelial origin.

The accompanying table shows the duration of survival of the micro-organisms in first "generation" cultures, experiments of August 17, October 10 and October 24, and the prolongation of this period by transplanting the cultures into fresh plasma; experiments of October 31 and November 7.

The examination of cultures fixed in Zenker's fluid, sectioned and stained with Giemsa's stain, shows that the period of survival of the micro-organisms corresponds to the length of survival of the cells of the culture. Initial multiplication of the micro-organisms takes place in situ in endothelial cells of blood vessels, and continues in wandering cells of the same origin.

In addition to the forms of the micro-organism previously described, filamentous forms are occasionally found resembling those of *Rickettsia prowazeki* as seen in infected lice.

With typhus we have not completed experiments beyond first "generation" cultures. Guinea pigs inoculated after eight, eleven and fourteen days incubation of the cultures have acquired typhus, as was proved by typical temperature reaction plus characteristic lesions in the brain or immunity.

Micro-organisms consistent with *Rickettsia prowazeki* have been found in sections of the brain cultures within large wandering amœboid cells; but their demonstration is attended with the same difficulties in these tissue cultures as in sections of fresh tissues with lesions. The examination of sections of the cultures shows that the surviving cells in these brain cultures take

Rocky Mountain Spotted Fever

Days in Vitro	1–5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24–27	28	29–34	35	36
Date — "Generation"																								
Aug. 17 1922 — I																								
Oct. 10 1922 — I		C22 +	C26 +		C27 +				C23 +	C28 +														
Oct. 19 1922 — I											C30 +				C31 −		C29 −							
Oct. 24 1922 — I										C32 −				C33 −										
— I										C34 +														
— II												C35 ‡	C36 +						C40 +					
Oct. 31 1922 — III																			C41 +					
— IV																								
— I										C38 +		C42 ‡	C43 +					C45 + / C46 −			C48 −			
— II																								
Nov. 7 1922 — III																					C47 ‡			
— IV																							C49 −	

EXPLANATION OF TABLE.

The arrows indicate transfers of the tissues or fragments thereof into fresh plasma.

‡ Indicates an atypical reaction followed by immunity to subsequent inoculation with spotted fever blood.

Guinea-pigs C22, C23, C27 were killed.

Guinea-pigs C26, C28, C30, C34, and C43 succumbed to the infection.

Guinea-pigs C35, C36, C38, C42, C45, and C47 survived the infection.

origin in blood vessels and meninges. Nerve cells and apparently neuroglia cells do not survive. The wandering amœboid cells we believe are of endothelial origin.

The well-known vagaries in duration of incubation periods and intensity of temperature reactions of typhus in guinea pigs render progress slow in these experiments. Our results so far prove that the micro-organism of typhus survives in first "generation" tissue cultures up to fourteen days.

These experiments with both diseases are being continued and extended, and in the case of typhus with other tissues than those of the central nervous system.

130 (2090)

The endothelial factor in anaphylaxis.

By W. H. MANWARING, R. C. CHILCOTE, and V. M. HOSEPIAN.

[*From the Laboratory of Experimental Pathology, Stanford University, California.*]

If the lungs of a normal dog are perfused with Locke's solution, followed by Locke's solution containing 0.25 per cent. to 1 per cent. horse serum, no recognizable pulmonary reaction takes place. The rate of perfusion flow remains constant on changing from Locke's solution to the dilute serum. The lungs collapse normally on releasing the tracheal clamp. No frothy fluid escapes from the trachea.

If the lungs of a sensitized dog are similarly perfused, marked pulmonary reactions occur. These reactions are:

(a) A 75 per cent. reduction in the rate of perfusion flow. This reduction reaches its maximum by the end of two minutes, with slight tendency to recovery after the third minute.

(b) An increase in the size and consistency of the lungs, with non-collapse on release of the tracheal clamp.

(c) The escape of large amounts of fluid from the trachea on releasing this clamp. If the perfusion is now continued,

fluid continues to pour out of the trachea almost as fast as it escapes from the efferent canula.

To our mind the increased capillary permeability thus demonstrated is the most significant feature of these reactions. We believe that increased specific capillary permeability will ultimately be shown to be the dominant fundamental factor in protein sensitization, to which all other anaphylactic phenomena are secondary. This view is in accord with clinical evidence.

131 (2091)

Types of canine anaphylaxis.

By W. H. MANWARING, R. C. CHILCOTE, and V. M. HOSEPIAN.

[*From the Laboratory of Experimental Pathology, Stanford University, California.*]

The typical anaphylactic reaction in dogs is characterized by a sudden, pronounced fall in arterial blood pressure. The pressure is usually reduced to about 25 mm. Hg. by the end of ninety seconds. This typical reaction is demonstrable in practically all dogs tested from eighteen to twenty-four days after intravenous horse serum sensitization. Recovery usually takes place in from one to two hours, depending upon the severity of the reaction.

We have recently encountered an example of a second type of canine anaphylaxis. This was in a dog tested during the seventh week of horse serum sensitization. In this dog no change in arterial blood pressure took place for four minutes after intravenous serum injection. The pressure then fell slowly and irregularly, death occurring in nine and one-half minutes.

The thorax of this dog was immediately opened. The lungs were found almost non-collapsible, but could be readily collapsed on pressure. This partial pulmonary fixation passed off in about fifteen minutes. The blood was rendered non-coagulable by the reaction, but slight hepatic changes were noted at autopsy, and no duodenal hemorrhage. The pathological findings resembled those of guinea pig anaphylaxis.

132 (2092)

The effects of complete extirpation of the hypophysis in the dog (Preliminary report).

By CLARENCE G. BROWN.

[*From the Hull Physiological Laboratory, University of Chicago, Chicago, Illinois.*]

A series of 66 dogs were operated through the oral route and the hypophysis removed by cautery. Five of these animals (Nos. 4, 30, 34, 35, 40) showed no clinical symptoms, and when killed for examination 96, 95, 95, 146 days after operation, no trace of hypophysis was found microscopically. One animal (No. 38) died 15 days after operation. This dog showed the symptoms of so-called cachexia hypophyseopriva. Autopsy showed absence of hypophysis, a blackening of the region about the infundibulum and a severe pneumonia. The pneumonia was probably an aspiration type and was diagnosed as such two days after the operation. Dog No. 52 remained alive for 259 days after hypophysectomy. This dog was adult at the time of operation, weighing 18 K. After hypophysectomy the dog gained 7 K., became sluggish, and somewhat somnolent. There was no demonstrable decrease in the size of the testes (weighed 31 g. at the time of death) and there was no microscopic trace of the hypophysis at autopsy.

Two dogs (Nos. 14 and 39) are still alive, two years and nine months and one year and nine months after hypophysectomy respectively. Dog 39 was adult at time of operation, weighing 5.8 K. After the operation this dog gained six kilos in weight, became extremely sluggish and somnolent (sleeps practically all the time), shows altered disposition (grouchy) and is devoid of sex interest (tested with bitch in heat). There is no gross decrease in the size of the testes as determined by external measurement. This animal showed polyuria and polydypsia for some time following the operation. Dog 15, a female pup, weighed 3 K. at time of operation. After the operation the pup gained four kilos, while the control gained six kilos. This hypophysectomized dog has an infantile appearance, is rather fat, and shows practically no development of the mammary

glands. She was caged with male dogs for a year and never became pregnant, but she is neither sluggish nor somnolent. The remainder of the series are eliminated because when killed for examination vestiges of hypophysis were found, or the dog died of pneumonia or meningitis soon after operation. The dogs with hypophysis remnants showed no symptoms of hypophyseal deficiency.

All the dogs (except No. 52) that died following the hypophysectomy operation, the cause of death was either meningitis or pneumonia (probably aspiration pneumonia). These dogs died within 3 to 10 days following the operation.

1. Completely hypophysectomized dogs may live indefinitely without showing any of the Fröhlich syndrome.

2. Some of the completely hypophysectomized dogs show Fröhlich's syndrome in varying degrees (retarded growth, adiposity, somnolence, loss of sex urge).

3. The fact that all completely hypophysectomized dogs do not exhibit the Fröhlich syndrome seems to indicate that the hypophysis itself is not the only factor involved in this malady. The obvious varying or uncontrollable additional factor is the injury to the base of the brain in these operations.

133 (2093)

The auto-hemolysin of paroxysmal hemoglobinuria.

By GEORGE M. MACKENZIE.

[From the Department of Medicine of the College of Physicians and Surgeons of Columbia University, and the Presbyterian Hospital, New York.]

The essential characteristic of the condition known as paroxysmal hemoglobinuria is the occurrence, as a result of exposure to cold, of hemoglobinæmia and hemoglobinuria, usually accompanied by a chill and rise of temperature. In the blood of these patients there is present an auto-hemolysin which is readily demonstrated by the simple procedure well known as the Landsteiner reaction. In its simplest form it consists simply

in chilling the patient's blood to 0° C. for 30 minutes, and then warming it to 37° C. In normal blood no hemolysis occurs after the blood has been warmed to 37°. More consistent results are obtained by using serum and a suspension of washed red cells, and by adding complement. This auto-hemolysin has been the subject of a number of studies which need not here be reviewed. The reaction has been shown to be an antigen-amboceptor-complement reaction with the characteristic that the hemolysin unites with the red cells only at a low temperature. It has also been shown that the combination of corpuscles and hemolysin occurring at low temperatures is at least partially broken up at higher temperatures, and that an iso-hemolysin demonstrated by chilling, as well as the auto-hemolysin, is present.

During the past three years we have had an opportunity to study the blood of three patients who are subject during the winter months to typical attacks of hemoglobinuria. Unless otherwise stated, the Landsteiner reaction has been done in our work by using 0.25 c.c. of serum, and 0.1 c.c. of a 5 per cent. suspension of washed red blood cells, and adding as complement 0.1 c.c. of pooled guinea pig serum diluted 1 : 10. The volume is made up to 0.5 c.c. with normal salt solution, and the tubes are then kept at 0° C. for 10 minutes, and in the water bath at 37° for 2 hours, when readings are made. Control serums and red cell suspensions were always from the same blood group.

In contrast with most sensitizing antibodies, this auto-hemolysin may show marked thermolability. In one of our cases 45° C. for 30 minutes destroyed the hemolysin so that it could not be reactivated by addition of fresh complement. The temperatures required to destroy the hemolysin in the other two cases were 47.5° C. and 55° C.

The demonstration of the presence of the auto-hemolysin is usually accomplished by chilling the serum-erythrocyte-complement mixture to 0° C., but obviously such a low temperature is not present at the site of the *in vivo* reaction. It seemed worth while, therefore, to determine the highest temperature at which the union of hemolysin and erythrocyte occurs. Two of the hemolysins sensitized at temperatures from 0° C. to 12° C.; the third did not sensitize above 8° C. It seems quite possible that the blood in the superficial capillaries may, in cold weather,

be chilled to 12° C., or even 8° C. It is not unlikely, therefore, that in the spontaneous paroxysm, the same mechanism operates as in the test tube reaction.

Yorke and Macfie[1] recently reported that hemolysis is greater if the erythrocyte-hemolysin-complement mixture is chilled only 5 to 7 minutes, rather than 30 to 60 minutes. We have confirmed this observation. Their explanation of this somewhat paradoxical phenomenon is that, with longer exposures at the low temperature, the complement is distributed among a larger number of sensitized cells with the result that each cell has less complement, and some cells have ineffective quantities. That this explanation is not entirely satisfactory is evident from the following experiment. To graded red cell suspensions from 0.5 per cent. to 50 per cent. constant amounts of serum and complement are added. The mixtures are chilled and then warmed in the usual manner. It is found that the amount of hemolysis increases with the percentage of the cell suspension, and that suspensions of 0.5 per cent. and 1.0 per cent. showed no hemolysis. With heavier suspensions there would presumably be a distribution of complement among a larger number of cells, and yet there is more hemolysis in the heavy suspensions. It therefore seems unlikely that the explanation of Yorke and Macfie for the greater hemolytic effect of short chilling is correct.

One of the interesting questions in paroxysmal hemoglobinuria is the relation of this condition to syphilis. About 90 per cent. of these patients as reported in the literature have had a positive Wasserman reaction. Many of them have been congenital syphilitics; others have had lesions of acquired syphilis, and some have had only serological evidence of syphilis. One of our three cases is a congenital syphilitic; another probably has syphilis of the liver, and the third has a history of syphilis but no demonstrable lesions at present. All three have positive Wasserman reactions, and it is of interest that the reaction in two of the three cases is extraordinarily strong. The highest titre was found in the congenital syphilitic, a girl of 11, whose Wasserman titration at .001 c.c. was: alcoholic antigen +++, cholesterin antigen ++++, larger quantities gave ++++ reactions with both antigens. The Landsteiner reaction was found

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[1] Yorke, W., and Macfie, J. W. S., *Brit. J. of Exp. Path.*, 1921, ii, 115.

to be negative in four congenital syphilitics without paroxysmal hemoglobinuria, all of whom had strong Wasserman reactions.

If the serum of the hemoglobinuric is exposed to an equal volume of a 100 per cent. suspension of his own erythrocytes at 0° C., and the mixture is centrigugalized cold, the supernatant serum is found to have lost the Landsteiner auto-hemolysin. It has been completely absorbed at the low temperature. Wasserman reactions done on absorbed and unabsorbed serum show an almost identical titre. It is, therefore, clear that the Landsteiner auto-hemolysin and the Wasserman reacting substance are distinct serological entities. The former may be removed without weakening the latter. This result harmonizes with the observation that of our three cases, the one having the highest titre of Landsteiner auto-hemolysin had the weakest Wasserman reaction.

Since the serum of the hemoglobinuric, in addition to the auto-hemolysin, contains a similar hemolysin for the erythrocytes of other individuals even of the same blood group, an attempt was made by absorption experiments to separate the auto-hemolysin from the iso-hemolysin. It was found that the red blood cells of other individuals, as well as the patient's own erythrocytes, absorbed out both the auto- and the iso-hemolysin. Presumably, therefore, they are inseparable and possibly identical.

It appears, therefore, that in this disease we are dealing with a thermolabile hemolysin which unites with its antigen only at a low temperature, that the union is more effective with chilling for only 5 to 10 minutes than with longer exposure to the low temperature; that complement is necessary for consummation of the reaction, that there is an intimate relation between this disease and syphilis, but that the substance upon which the diagnostic test tube reactions depend are not identical.

Abstracts of Communications.

Pacific Coast Branch

Thirty-sixth meeting.

San Francisco, California, February 14, 1923.

134 (2094)

An anaerobe from the mouth cavity of man and rabbits morphologically suggesting B. pneumosintes.

By W. L. HOLMAN and F. H. KROCK.

[*From the Department of Bacteriology and Experimental Pathology, Stanford University, California.*]

One of us (W. L. H.) reported in 1919 the isolation of a minute anærobe out of material from the mouth of five consecutive persons not suffering from influenza, in Pittsburgh, Pennsylvania.

The material was cultured on cooked meat and other media, and the bacterium attracted attention because of the abundance of gas it produced in cooked meat medium, but more particularly on account of its small size and the chance of its being confused in direct smears and mixed cultures with *B. influenzæ*. It was thought at the time to be closely related to the *Staphylococcus parvulus* of Veillon and Züber, but the descriptions of this gram negative anærobe are too meager to permit complete identification. An anærobe, which we considered to be the same, was reported at the meeting of the Association of Pathologists and Bacteriologists in May, 1922, as "A very small anærobe giving gas in tissue." The site of infection in the neck of this patient was in direct communication with the mouth cavity, and it may well have been an accidental contamination or something more important. A non-hemolytic streptococcus was found associated with it.

Anærobes which we consider as identical with the six strains mentioned have been readily isolated from the mouth cavity of four of us in the laboratory at Stanford University, California. It would appear that this anærobe, or very closely related forms,

is commonly if not always present in the oral cavities of human beings.

The anærobe is very small, well under 0.5 micra long, by a little less in width. (Measurements under a half micron are not very accurate). It is smaller than *B. prodigiosus, B. bronchosepticus* and *B. influenzæ.* Illustrations of *B. pneumosintes,* as given by Olitsky and Gates in their earlier reports, indicate its size. This coccoid bacillus is non-motile, gram negative, occurs singly, often in pairs, and in irregular groups, depending on the medium, and is strictly and persistently anærobic. In Veillon agar shakes, its colonies, never growing nearer than 1 cm. to the surface, vary with the dilution used, and are remarkably uniform in any given tube. The size varies from very tiny colonies when crowded to large disks or compound disks when widely isolated. The same variation in size is true of surface colonies. They remain individual even when crowded, are raised, with a clearer sharp border and of a greyish white color. It is rather restricted in its test tube biological characters. In Veillon agar (containing sugar) gas production may be, and often is, absent. On occasions it may produce abundant gas. The conditions favoring or interfering with the demonstration of gas has not been determined, but it would appear to be independent of sugar content. The same is true in fluid media, using our (W. L. H.) modification of the Hall anærobic tube. There is no indication of acid production.

The gas production in cooked meat medium is constant and abundant, and is the outstanding test tube biological feature. The gas contains traces of hydrogen sulphide, but its analysis is not completed.

We have been able to pass this minute anærobe through a Mandler filter tested against *B. pyocyaneus,* but which allowed *B. prodigiosus* to go through under 20 pounds pressure. We are at present developing a method for more accurately testing filters before using them for bacterial filtration, following the method given by Bullock and Craw, and by Ferry. The work of Ferry on the filtration of *B. bronchisepticus* is important in this connection.

We have further been able to isolate this same tiny anærobe from the mouth cavity of two normal rabbits.

We consider this bacterium of importance for the following reasons: (1) It may easily be confused with *B. pneumosintes*

by its morphological appearance and its manner of growth. The failure of *B. pneumosintes* to produce gas does not help in the differentiation since the bacterium we are describing frequently fails to show gas. I do not believe the cooked meat medium was used by Olitsky and Gates. (2) It is filterable through tested filters. Olitsky and Gates do not give any method by which their filters were tested. (3) It is found in the oral cavities of man and rabbits, and thus could lead to confusion with *B. pneumosintes* from these sources. (4) Comparable experiments to those done by Olitsky and Gates will have to be carried out to determine whether this common, extremely small anærobe will alter the blood picture after intratracheal injection, or lower the resistance of the lung to secondary invasions by other common micro-organisms of the respiratory tract, or will show any serological or other relationship to *B. pneumosintes*.

135 (2095)

Anaphylactic reactions in isolated canine organs.

By W. H. MANWARING, R. C. CHILCOTE, and V. M. HOSEPIAN.

[*From the Laboratory of Bacteriology and Experimental Pathology, Stanford University, California.*]

If the isolated organs of horse serum sensitized dogs are perfused with Locke's solution containing 0.5 to 1 per cent. horse serum, the following reactions are observed:

(a) *Hind quarters*: Slight increase in perfusion resistance, reducing the rate of perfusion flow about five per cent. No demonstrable edema, except on genitalin (female).

(b) *Intestines*: Increased perfusion resistance, reducing the perfusion flow about twenty-five per cent. Increased peristaltic movements; distinct edema of intestinal wall; increased volume of intestinal contents.

(c) *Liver*: Increased perfusion resistance, reducing the perfusion flow about twenty-five per cent. Distinct hepatic edema.

(d) *Lungs*: Marked increase in perfusion resistance, reducing the perfusion flow fully seventy-five per cent. Marked pulmonary edema; non-collapse of lungs on releasing the tracheal clamp.

The reactions in the intestines, liver and lungs are qualitatively similar to the histamine reactions previously reported.[1] The reactions in the hind quarters, however, differ from the histamine reactions: (a) in the absence of the marked edema characteristic of the histamine perfusion, and (b) in the substitution of a slight vasoconstriction for the marked histamine vasodilation.

If reactions similar to those observed on blood-free perfusions of isolated organs take place during anaphylactic shock in the intact animal, one can readily understand why the acute fall in arterial blood pressure, the characteristic feature of canine anaphylaxis, does not take place in dehepatized dogs. Peripheral vasoconstriction (intestines, hind quarters) would tend to increase the arterial blood pressure in these animals, while the reduced blood volume from edema would tend to decrease this pressure. The combined action of these two factors might readily leave the blood pressure unaltered. The pulmonary vasoconstriction is presumably compensated for by an increased strength of the myocardial contractions.

Defibrinated blood perfusions will be reported later.

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1 Manwaring, W. H., Monaco, R. E., and Marino, H. D., PROC. SOC. EXP. BIOL. AND MED., 1922, xx, No. 5.

ABSTRACTS OF COMMUNICATIONS.

Minnesota Branch

Tenth meeting.

Minneapolis, Minnesota, February 14, 1923.

136 (2096)

Effects of electricity on noctilucca.

By E. P. LYON

[*From the University of Minnesota, Minneapolis, Minnesota.*]

I.   A brief direct current or a single break induction shock causes the protoplasm to pull away from the cell wall.   The effect is usually cathodal; sometimes anodal, particularly when the aboral part of the cell is toward the anode.   Sometimes the effect is seen simultaneously at both poles.

The phenomenon gives the impression of contraction and breaking of the strands of protoplasm attached to the cell wall or pellicle.   A clear area looking like a blister filled with liquid is formed, the shape being dependent on the extent of protoplasm pulled away from the wall and the resulting disturbance of protoplasmic stresses.   If the bleb is small, it may be pinched off later without further deformation of the organism.   If the bleb is large, gradually the protoplasm pulls loose all the way around, the strands being seen to give way one or a few at a time.   Finally the whole protoplasm shrinks into a shapeless mass around the oral end.   Recovery is sometimes possible, but probably involves the formation of a smaller cell with new cell wall.

It appeared certain that the strands are solid structures along which a considerable tension is exerted.

A short tetanizing current gives blisters at both poles.

II.   If such a dye as phenolsulphonpthalein was placed in the sea water between the cells of a dense culture, the color could be observed for fifteen minutes or more.   But if a break shock or short tetanizing current was passed, the color disappeared at once.   The cell juice of *Noctilucca* is strongly acid (Ethel Brown Harvey, *Carnegie Reports,* Marine Biology, 1917).   Stimula-

tion caused increased permeability and the acid diffused out. This effect could not be obtained with currents too weak to cause the bleb formation previously described.

III.   Mrs. Harvey states that the tentacle coils at the make remains coiled while the current is passing and relaxes at the break.   This is true for certain individuals and certain positions of the organisms relative to the current direction, but not for all.   There is considerable variety of detail for which the reader is referred to a fuller report to be published later.   When the current is from aboral to oral the usual effect is as follows: Strong contraction of tentacle at make; held contracted nearly to full extent while current is passing; slight contraction (sometimes none) at break followed by marked relaxation.   When current is oral to aboral: extreme relaxation at the make (sometimes preceded by a slight instantaneous contraction); held relaxed while current is passing; strong contraction at break, followed by return to normal position.   Often spontaneous to and fro movement was seen either in the strongly contracted or strongly relaxed tentacle while current was passing.   These effects of the current seem explicable on the basis of electrotonus.

137 (2097)

Observations on the assay and factors influencing the quality of digitalis.

By E. L. NEWCOMB.

[*From the University of Minnesota, Minneapolis, Minnesota.*]

A method for assaying digitalis and other drugs of this group by the use of cats was outlined.   The procedure was practically identical with the Hatcher-Ouabain Method, as published in the *American Journal of Pharmacy,* 1910, but differed in the important point of completing the test with the digitalis to be standardized, rather than with a standard Ouabain solution. Results by this method, it was stated, were comparable with those obtained with Ouabain, and as accurate as the use of frogs or guinea pigs for determining toxicity.   Regulating the dose

and rate of flow in proportion to the weight of the animal was held to be important, if satisfactory results were to be obtained. The use of the cat was held to possess distinct advantages in that it afforded the analyst the opportunity of recording the exact action of the preparation upon the heart. It was held to be a possibility that further work might result in a method for measuring the total therapeutic activity, as well as the toxicity.

Results were submitted showing that the first year's growth digitalis leaves as produced at the University of Minnesota during 1922 were the equal in every way with respect to therapeutic value to leaves collected from second year's growth, provided the long petioles which frequently develop during the first year's growth were not included in the drug. The petioles, it was pointed out, represented 40 per cent. of the weight of the entire dried leaf. The general practice is not to collect these long leaf stalks. The leaf stalks or petioles were stated to be only about one-quarter as rich in therapeutic constituents as the lamina.

138 (2098)

Experimental goitre and iodin in natural waters in relation to distribution of goitre.

By J. F. McCLENDON and AGNES WILLIAMS.

[*From the Laboratory of Physiological Chemistry, University of Minnesota, Minneapolis, Minnesota.*]

White rats of 30 gram weight were placed on a diet containing about or less than .006 milligram of iodin in 50 grams of dry foodstuff in addition to distilled water. Controls were placed on the same diet except for one day a week when they drank water containing .01 per cent. iodin. At the end of about three months those receiving the iodin had thyroid glands one-half to two-thirds the weight of those receiving no iodin.

The United States is divided into four zones based on the

number of goiters too large for wearing military collars per 1,000 drafted men. The amount in parts per billion of iodin in drinking water is also given:

ZONE I—Goitre, 1-30. Iodin, 0.1-1. Washington, Oregon, Wyoming, Montana, parts of Utah, Minnesota, Wisconsin, and Michigan.

ZONE II—Goitre, 5-15. Iodin, .015-1.2. Nevada, Colorado, North Dakota, Iowa, Ohio, Indiana, West Virginia, parts of California, Utah, South Dakota, Minnesota, Wisconsin, Michigan, Pennsylvania.

ZONE III—Goitre, 1-5. Iodin, .06-9. Nebraska, Kansas, Missouri, Illinois, Kentucky, Tennessee, parts of California, Arizona, New Mexico, South Dakota, South Carolina, North Carolina, Virginia, Maryland, Pennsylvania, New York.

ZONE IV—Goitre, 0.1. Iodin, 1.4-9.7. Texas Indian Territory, Arkansas, Louisiana, Mississippi, Georgia, Alabama, Florida, parts of Arizona, New Mexico, South Carolina, and a strip along the Atlantic seaboard.

These zones run from east to west but are diverted southward in the mountainous regions and northward in the Great Plains area which contained a large salt lake centering in Kansas during the Permian period. The goiter-free southern states were submerged beneath the sea even as late as the Pliocene period.

In order to determine the iodin in drinking water quantitatively very large samples are necessary, in fact the smallest sample from Zone 1 must be at least 25 gallons; Zone II, 15 gallons; Zone III, 10 gallons; Zone IV, 5 gallons. Even with such sized samples we often fail to find sufficient iodin for strictly quantitative analysis. It is hoped that interested persons will assist in this public health measure by forwarding samples, in return for which we will send them the analysis. Place about a teaspoonful of soda in a large dishpan, add water and evaporate until the required total volume has been added and reduced to about one liter, filter into a beaker and evaporate to as small volume as practicable and transfer to an evaporating dish; evaporate to dryness without burning; scrape out the dry sample and send it to us for analysis.

139 (2099)

The presence of anti-ophthalmic vitamin and the absence of anti-richitic vitamin in dried spinach.

By J. F. McCLENDON and CECILIA SHUCK.

[*From the Laboratory of Physiological Chemistry, University of Minnesota, Minneapolis, Minnesota.*]

Steenbock has noted the decrease or disappearance of the anti-rachitic substances in leaves, and MacCollum has distinguished between the anti-rachitic and the anti-ophthalmic vitamin A. Experiments on spinach have covered a period of two years and at the beginning of this period the dietary ingredients were procured in large quantities and stored in the dry state in galvanized iron containers to insure uniformity of product covering all of these experiments. Changes might take place due to ageing, but the experience of others shows that such change would be the disappearance of a vitamin and never the production of a vitamin.

In very numerous experiments spinach has demonstrated no anti-rachitic property except the small effect of the phosphate it contains, which is usually offset by the increased growth. It is not possible to feed as much roughage to a rat as to a true herbivorous animal. If 50 per cent. of the diet is made of leaves, such as alfalfa meal or dried spinach, rats die in a relatively short time. Spinach was fed in quantities varying from 0.01 per cent. to 75 per cent. of the diet and in no case was any appreciable anti-rachitic effect noted. On the other hand, 0.1 per cent. showed a definite retarding of ophthalmia (keratomalacia) and a slightly greater amount was able to prevent ophthalmia over a relatively long period. Very advanced cases of ophthalmia were cured in six days by feeding half a gram of spinach a day. In case the lens of the eye had already popped out the spinach did not cause it to go back in the eye in six days, but apparently the cornea was regenerated in order to protect the extruded lens and the eye assumed a healthy even though a somewhat deformed condition. In case the lens had not popped out of the eye, the eye in every way appeared normal after the spinach diet.

140 (2100)

Adsorption hemolysis.

By R. G. GREEN and C. W. STOMBERG.

[*From the Department of Bacteriology, University of Minnesota,
Minneapolis, Minnesota.*]

This report concerns the mechanism of action of certain hemolytic agents which have the property of lowering surface tension, examples of which are castor oil soap and saponin. These belong to a general class of substances which reduce the surface tension of their solutions according to the formula

$$y = \frac{\gamma_1 - \gamma_2}{e^{kx}} + \gamma_2$$

y = surface tension at any concentration.
x = concentration of (hemolytic) solute.
γ_1 = surface tension of liquid solvent.
γ_2 = surface tension of saturated surface.
k = constant, the value of which depends upon the relative distribution of the solute in the body and surface of the solvent.

In these preliminary investigations erythrocytes from sheep have been placed in normal salt solutions containing known concentrations of the hemolytic substance, the time of hemolysis being carefully measured by means of a stop watch and a well defined end point. The surface tensions of solutions of hemolytic substance in the concentrations used were also measured. It was found, as is evident from the formula, that as the concentrations of hemolytic substance increased, the surface tension of the solution dropped from that of the pure solvent to the value always obtained for a very concentrated solution of the substance.

It has been observed that as the concentration of the hemolytic substance increased, the time of hemolysis decreased until it reached a fairly constant value. The significant observation is that the time of hemolysis and the surface tension reach a fairly constant value at the same concentration of hemolytic substance. As the surface tension is dependent upon surface concentration we may say that the time of hemolysis decreases as long as the surface can adsorb more hemolytic agent, and that when the surface is relatively saturated the time of hemolysis is no longer

decreased by a further increase in solution concentration. Thus, it would appear that hemolysis by these agents is concerned with surface concentration rather than with solution concentration. If we assume that the time of hemolysis is inversely proportional to the surface adsorption the time of hemolysis can be expressed by the equation

$$t = amx^{-1/n} \text{ when}$$

$t = $ time of hemolysis,
$x = $ concentration of hemolytic substance,
$m = $ constant dependent upon the extent of adsorbing surface.
a and n are impirical constants.

This equation, which is the reciprocal of the adsorption formula, is graphically similar to the plotted values from experimental data which further indicates that hemolysis by surface tension reducing substances is a matter of surface concentration or an adsorption phenomenon.

141 (2101)

The fragility of erythrocytes treated with soap and saponin.

By R. G. GREEN and R. D. EVANS.

[From the Department of Bacteriology, University of Minnesota, Minneapolis, Minnesota.]

It was shown by preliminary experiments with hemolytic agents which were active in reducing surface tension that in very low concentration the time of hemolysis was greatly prolonged. The work here reported has been done to determine if a surface concentration of these substances on red blood cells could be demonstrated when the surface concentration was insufficient to cause hemolysis.

Having determined what concentration of castor oil soap and saponin in 0.9 per cent. NaCl would not hemolyze erythrocytes in a number of hours, red blood cells were placed in solutions of the determined concentration and allowed to stand for varying lengths of time. These cells were then removed and resuspended in 0.9 per cent. NaCl and time-fragility tests performed. We have found that the adsorption of castor oil soap on the sur-

face of red blood cells in concentration insufficient to cause hemo-
lysis greatly increases the time of hemolysis in a time-fragility
test. When saponin is adsorbed by erythrocytes in a non-hemo-
lytic concentration the time of hemolysis by hypotonic saline
solutions is decreased. This increased and decreased fragility
demonstrates the presence of the hemolytic agent definitely in
connection with the red blood cell surface, and bears further
evidence that hemolysis by agents of this type is an adsorption
phenomenon. The difference of castor oil soap and saponin in
producing a decrease and increase in fragility would seem to
indicate that there is a difference of mechanism of hemolysis
even in those hemolytic agents acting by surface adsorption.
We have found a great difference of susceptibility to the action
of soap and saponin in the case of human, sheep and bovine
erythrocytes, and a similar variation is also observed in our
time-fragility tests.

142 (2102)

The fragility of erythrocytes in obstructive jaundice and pernicious anemia.

By ROBERT G. GREEN (by invitation).

[*From the Department of Bacteriology, University of Minnesota, Minneapolis, Minnesota.*]

Our previous work demonstrating the action of small amounts
of hemolytic substances as castor oil soap and saponin in de-
creasing and increasing the fragility of red blood cells would
seem to have some bearing upon the fragility test as used in
medical diagnosis. The preliminary work to determine the rôle
of adsorption hemolysis in clinical conditions is here reported.

Bile from various animals has been used as a hemolytic
agent for various animal erythrocytes and we have found
its action to be very variable in different samples. The
surface tension of bile solutions has also been studied and its
surface tension reducing property is likewise very variable.

There is a very marked relation between the time of hemolysis and relative surface tension. Some samples of bile have shown a decrease in surface tension upon dilution with salt solution and coördinate with this has occurred a decrease in the time of hemolysis. When erythrocytes are treated with non-hemolytic concentrations of bile, the fragility of the cells are sometimes greatly decreased and sometimes increased. This corresponds with clinical findings and adsorption of bile elements on the surface of the red blood cells appears responsible for the changes in fragility observed.

In pernicious anemia a decreased or normal fragility of red blood cells is found. W. P. Larson suggested some years ago to the author that the hemolytic agent was probably a substance with a marked surface tension reducing property. In our experiments we determined carefully by means of a time-fragility test the fragility of the cells from pernicious anemia and the fragility of normal human cells from a blood of the same group. The normal cells were then treated with the serum from the pernicious anemia patient for varying lengths of time. These treated cells were then washed several times in salt solution and their fragility again measured. It was found that the treated normal cells showed a marked decreased fragility and in this respect appeared identical with pernicious anemia cells. Dilution of the pathologic serum gave similar results in varying degrees. These experiments lend some additional evidence to the view that the hemolytic agent is present in the serum. If it is the hemolytic agent which is responsible for the decreased fragility, the adsorption upon normal cells becomes a means of identification of the hemolytic factor in further experimental work.

The results reported from this laboratory upon hemolysis by soap, saponin and bile tend to show that the clinical fragility test is no indication whatever of a corresponding condition of the red blood cells. An erythrocyte partially hemolyzed by bile or by castor oil soap has an increased resistance to hemolysis by hypotonic salt solution.

Thus, any change in the fragility of erythrocytes in a clinical test, whether increased or decreased, must be interpreted as an indication that the red cells are being subjected to an accelerated hemolysis.

143 (2103)

Differential counting of living and dead cells of bacteria.

By ARTHUR T. HENRICI.

[*From the Department of Bacteriology and Immunology, the University of Minnesota, Minneapolis, Minnesota.*]

By means of the procedure here described it is possible to determine with considerable accuracy the number of bacterial cells in a suspension and at the same time to determine the size and form of the cells and to differentiate the living and dead cells. The method makes use of the principle of the Breed and Brew[1] method of counting bacteria in milk, and the negative staining method of Benians.[2]

A measured quantity of bacterial suspension is mixed thoroughly with an equal quantity of 2 per cent. aqueous Congo red solution; the mixture is allowed to stand ten minutes. After again shaking the mixture, 0.01 c.c. is removed by means of a capillary pipette of that capacity and discharged on to a clean slide which has been clamped to the table over a piece of white paper on which a 2 cm. square has been ruled. By means of a stiff wire the drop of liquid is spread as evenly as possible over this area. After it has become thoroughly dry the slide is immersed a moment in a 1 per cent. solution of hydrochloric acid in 95 per cent. alcohol. This turns the dye blue and also fixes the film. If covered with a layer of cedar oil the slides will keep indefinitely, but if exposed to the air they fade considerably.

Cells which were alive at the time of staining are unstained and appear as white spots on a blue ground. While the cells themselves may shrink considerably after fixation and drying, a number of comparative measurements have shown that the clear space in the film faithfully reproduces the size and form of the living wet cells. With favorable material flagella may be demonstrated by this stain.

Seiffert[3] has observed that when bacteria are suspended in a weak Congo red solution and examined in hanging drops that

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1 Technical Bulletin No. 49, New York Agricultural Experiment Station.
2 *British Medical Journal*, 1917, ii, 722.
3 *Centralbl. fur. Bakt.*, etc., Abt. I, Orig., 1922, lxxxviii, 151.

the dead cells are stained whereas the living cells are not. The differentiation by this method is, however, not so sharp as when Benians' technique is used. In the latter case the dead cells appear a distinct blue color, usually deeper in tint than the surrounding film of dye. I have found that with suspensions killed by heat the staining is not intense immediately after killing, but that the cells stain more deeply if allowed to stand several hours after heating; and that they stain more readily after heating to 60° than when heated to 100°. When killed and preserved in formalin the cells do not stain; but dead cells preserved in formalin retain their staining properties. It would seem that autolysis must commence before the cells can stain.

The metachromatic granules of some diphtheroids and the sporogenous granules of some bacilli are stained by the Congo red even in the living cells. The differentiation of the living and dead cells can not, therefore, be attributed to changes in the permeability of the cell membrane. The stain not only colors the protoplasm of the dead cells but is also concentrated in the film about them. It is possible that the staining may be explained by a loss or change of electrical charge in the cells.

The films dry rather unevenly, being denser in the middle. It is best to prepare a number of slides and to choose for counting those which show the most uniform films. At least five slides should be counted to compensate for the uneven distribution. The counting is done by means of an eye-piece micrometer ruled in squares and calibrated against a stage micrometer. With suspensions of ten million per c.c. or over I count twenty fields of .01 sq. mm. each from each of five slides. The average deviation of such counts is usually less than 10 per cent. A growth curve obtained by this method of counting was much smoother than those obtained by other methods. A series of comparative counts of a yeast cell suspension by the method described and by the use of a counting chamber of the Helber type showed that the two procedures were about equally accurate. But with small bacteria the cells can be seen so much more distinctly in the negatively stained film that a much larger number can be counted without fatigue or eyestrain than is possible with the counting chamber; and I believe that the method will prove correspondingly more accurate with such organisms.

Broth and peptone solution precipitate the Congo red. The method can not be used with such cultures unless they are first

centrifuged and the sediment is resuspended in a measured volume of water or salt solution. This is not true of the so-called synthetic media which contain no protein substances.

144 (2104)

Demonstration of an instrument for taking repeated blood pressures in rabbits, with report of some experiments.

By H. C. ANDERSON (by invitation).

[*From the University of Minnesota, Minneapolis, Minnesota.*]

In connection with a study of renal insufficiency in rabbits,[1] it became desirable to take a series of blood pressures on the animals. An instrument has been perfected by means of which the blood pressure can be taken in the central artery of the ear. The instrument is composed essentially of three parts; namely, a pressure piece, a "U" tube containing mercury, and a rubber bulb with which to make pressure.
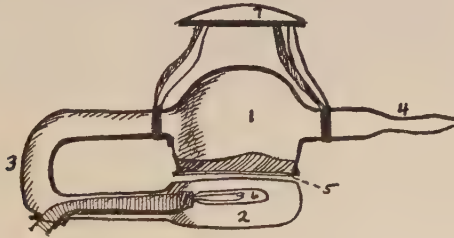
The pressure piece is made of glass. It is composed of an open cup (1) (see drawing) an apposing smooth, slightly convex stage (2), a connecting curved arm (3), and a short glass point (4) by means of which the cup may be connected with the "U" tube. The mouth of the cup is covered by a rubber membrane (5) which, when pressure is made within the cup, bulges against the stage. The rabbit's ear is slipped between the stage and rubber membrane. The stage contains a light

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[1] To be reported at a later date.

(6). Above the cup a lens (7) is placed for the purpose of magnifying the stage.

In use, pressure is made within the cup by means of the rubber bulb. The membrane presses against the ear vessel and the pulsations are observed by means of the light transmitted from within the stage. When pulsations cease, the pressure is read in millimeters of mercury on the "U" tube.

Factors Which Vary the Pressure

Age. It was found that age has a definite influence on the pressure. Unless the rabbit is full-grown the pressure is inclined to be low.

Excitation. Anything which excites the animal increases the pressure. Six rabbits which were mildly excited by lifting them from the table by the ears and gently rubbing the ribs showed an average rise of 13.3 mm.

Heat. Warming the ear by any means, or rubbing it with the fingers, causes a definite rise which may be as great as 20 mm. This seems to be because of the vaso-dilation.

Exercise. Almost any small amount of exercise raises the pressure. The ten readings were taken in rapid succession. At the third reading the animal stood up, stretched, and lay down again. As a result the pressure promptly rose 10 mm., but immediately returned to its former level.

The pressure readings must always be made at the same point in the vessel. The pressure decreases as the vessel becomes smaller.

Results obtained. The average systolic readings on various individuals lie largely between 76 and 87 mm. Isolated readings may run 90 or somewhat above and as low as 70. The averages on nine rabbits, representing between 500 and 600 readings, are as follows:

Rabbit 21.........83 mm.	Rabbit 25.........78 mm.	Rabbit 28.........83 mm.
Rabbit 23.........85 mm.	Rabbit 26.........82 mm.	Rabbit 29.........79 mm.
Rabbit 24.........87 mm.	Rabbit 27.........82 mm.	Rabbit 30.........79 mm.

Action of adrenalin. Subcutaneous injection of adrenalin results in a typical rise of pressure beginning from 10 to 20 minutes after the injection.

Sources of error. The greatest source of error seems to be in the changes of vessel diameter due to vaso-motor stimula-

tion. This is controlled largely by using ear muffs to prevent heat radiation.

Other sources are excitement and movement on the part of the animal.

Conclusions.

1. The blood pressure in the central artery of a rabbit's ear under properly controlled conditions ranges from 75 to 90 mm. of mercury.

2. The figures are sufficiently consistent to allow experimental study of the blood pressure.

3. Excitation, exercise and any stimulation tending to dilate the vessels locally increases the pressure above basal figures.

4. Injection of adrenalin shows the typical rise.

Abstracts of Communications.

Western New York Branch

Fifth meeting.

Syracuse, New York, February 17, 1923.

145 (2105)

The production of experimental anemia with symmetrical di-isopropyl-hydrazine hydrochloride and related compounds.

By MEYER BODANSKY and HENRY C. HARTMAN (by invitation).

[*From the University of Texas, School of Medicine, Galveston, Texas.*]

In its physiological behavior, phenylhydrazine differs very markedly from hydrazine in that it is extremely destructive of red corpuscles. This effect may perhaps be attributed to the phenyl group in the phenylhydrazine molecule. That alkyl substitution products of hydrazine, such as symmetrical di-isopropyl-hydrazine,[1] may produce a very pronounced anemia, is shown by the data in the following table:

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[1] This compound was synthesized by H. L. Lochte in the laboratories of J. R. Bailey and W. A. Noyes (*J. Am. Chem. Soc.*, 1921, xliii, 2597). The authors are indebted to Dr. Lochte for supplying them with this compound.

DOG 2-1, MALE, WEIGHT 12.9 KILOS.

Day.	R. B. C. millions.	Hemoglobin per cent.	Color index.	Remarks
1	5.40	83	0.77	Administered subcutaneously 0.258 grams symmetrical compound.
3	5.20	83	0.80	Marked lipemia.
4	3.80	77	1.01	Dog very sick.
5	3.60	66	0.92	Dog somewhat improved.
7	2.30	44	0.96	Dog much improved; 0.120 gram of compound administered.
8	2.20	33	0.75	Dog received 0.240 grams of compound.
10	0.92	28	1.52	
11	1.05	25	1.19	

As in the case of phenylhydrazine, the isopropyl derivative produces hypertrophy of the spleen and very marked hyperplasia of the bone marrow. Symmetrical di-isopropyl-hydrazine also resembles hydrazine in its effect upon the liver;[2, 3] fatty degeneration being produced after the administration of relatively small doses.

146 (2106)

Further studies of the relative rates of absorption of drugs from the lymph sac and the muscles of the frog.

By C. D. HIGLEY and M. S. DOOLEY.

[*From the Syracuse University Medical School, Syracuse, New York.*]

Previously it has been shown that members of the digitalis group as measured by their intensity of action upon the heart, are absorbed more rapidly and evenly from intramuscular injections than from the lymph sac. Strychnine was also found to be more rapidly absorbed from the muscles. These facts led us to suggest that in the official assay of the digitalis group of drugs the substitution of intramuscular injections be made. The present paper deals with further studies of this question.

2 Underhill, F. P., and Kleiner, I. S., *J. Biol. Chem.*, 1908, iv, 165.
3 Wells, H. G., *J. Exp. Med.*, 1908, x, 457.

Figure I. Epinephrin effects upon the frog's pupil.
TABLE I.

|	Lymph sac.	Intramuscular	Per cent.
Normal diameter	2.10 mm.	2.14 mm.	
Latent period	3.10 min.	1.08 min.	187
Period of increasing pupil diameter..	6.85 min.	14.00 min.	104.3
Maximum increase of pupil diameter	1.55 mm.	1.55 mm.	
Duration of maximum pupil diameter	20.90 min.	27.30 min.	30.6

TABLE II.

	Lymph sac	Intramuscular	Per cent.
Normal diameter	2.37 mm.	2.30 mm.	
Latent period	1.13 min.	0.75 min.	33
Period of increasing pupil diameter..	4.56 min.	3.64 min.	25.2
Maximum increase of pupil diameter	0.26 mm.	0.28 mm.	
Duration of maximum pupil diameter	5.38 min.	8.68 min.	61.3

Fig. II. -Epinephrin effects upon the frog's pupil.

Avg. Curves Series I.

Minutes
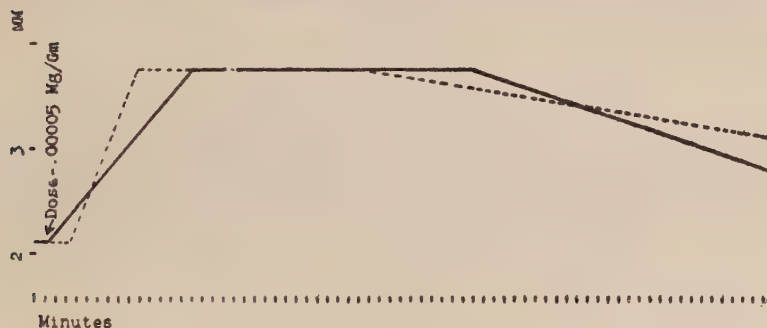
Avg. Curves Series II.

Minutes
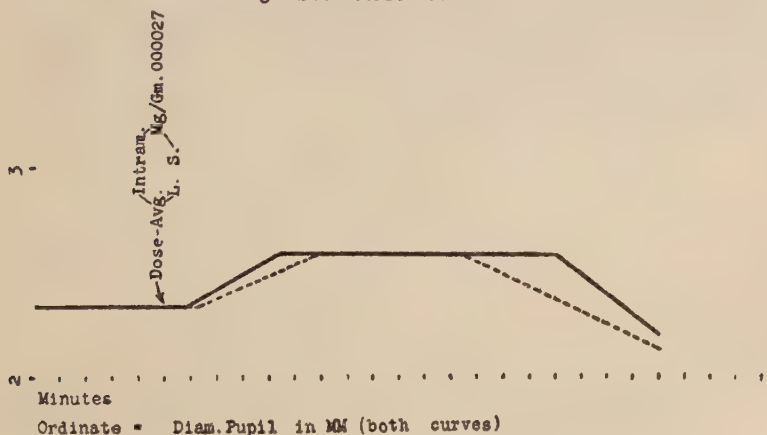
Ordinate = Diam. Pupil in MM (both curves)

Epinephrin has been chosen for study because of the readily measurable changes it induces in the diameter of the frog's pupil. The time from injection of the drug to beginning dilatation has been designated as the latent period and has been made the basis of comparison of the rates of absorption by the two methods. As the tables and curves (Figures I and II) show the latent period after intramuscular injection is always shorter. It is felt that this finding further substantiates the above results.

147 (2107)

Some effects of morphine upon respiratory reflexes.

By M. S. DOOLEY and GEORGE B. ANDREWS.

[*From the College of Medicine of Syracuse University, Syracuse, New York.*]

In some unpublished experiments it was found that morphine not only does not diminish the Hering-Breuer reflex, but, on the other hand, actually renders it more prominent, especially the inhibitory phase of it. This appears to be directly opposite to its effect upon other respiratory reflexes such, for instance, as the cough reflex and certain dyspnœas of reflex origin which are caused to disappear under morphine action. But, for reasons which can not be stated here, we believe that the cough reflex, especially, belongs to a different category and is not to be regarded as a simple reflex and, hence, responds differently. The experiments here reported were devised in an attempt to shed further light on this question.

It was stated above that especially the inhibitory phase of the Hering-Breuer reflex is exaggerated by the action of morphine. This has led us to examine another purely inhibitory respiratory reflex as to its behavior under the influence of the drug. To do this we have taken advantage of the fact, discovered by Myer, that the active expiration in the fowl is always inhibited by any effective electrical stimulation of the central end of the vagus nerve. For a given strength of stimulus morphine greatly prolongs this inhibitory reflex rather than diminishing or abolishing it. It is thus seen that this reflex reacts toward morphine as does the Hering-Breuer and not as does the cough reflex.

SCIENTIFIC PROCEEDINGS

148 (2108)

Food accessory substances and the nitrite bacteria.

By T. J. MURRAY.

[*From the Department of Bacteriology, Rutgers College, New Brunswick, N. J.*]

In the isolation of nitrifying bacteria, soil is inoculated into a synthetic solution containing $(NH_4)_2 SO_4$ as a source of nitrogen for the "nitrite" bacteria and into one containing $NaNO_2$ for the "nitrate" bacteria. The solutions are incubated and when a test for nitrites is found in one case or a test for nitrates in the other, a small amount of the liquid is transferred to a new synthetic solution. This transferring is continued for some time. Then the material is plated out on silica jelly containing suitable inorganic salts. From the colonies developing, inoculations are made into synthetic solution and if nitrites are formed in the one containing $(NH_4)_2 SO_4$ a "nitrite" bacteria has been isolated or if nitrates are formed where $NaNO_2$ is the source of nitrogen a "nitrate" bacteria has been isolated.

Great difficulty was experienced in isolating cultures by the above procedure. At the beginning, in no case where inoculations from the colonies on silica jelly were made into the synthetic solution, were positive tests subsequently obtained. If, however, a solution giving a positive test were centrifuged and the sedi-

ment inoculated on silica jelly by means of a loop and from the colonies that developed, inoculations were made into synthetic solutions, positive tests would be obtained. This applied to both the "nitrite" and the "nitrate" organisms. Now if these positive cultures were inoculated on nutrient agar, growth would appear. This growth would not cause nitrification, showing the cultures in question were not pure. In one experiment the synthetic solution for nitrate bacteria which gave a positive test was centrifuged and inoculated on a gypsum block partly immersed in the synthetic solution. From the growth later obtained, dilutions were made and plated on silica jelly. Ten colonies were picked. Five grew on nutrient agar and five did not. The ten cultures were inoculated into the synthetic solution for nitrate formation. The five that did not grow on nutrient agar never developed any nitrates, the other five that were shown contaminated by the growth on nutrient agar all gave positive tests for nitrate after incubation. The procedure has been varied in many ways, but whenever and by whatever method used, when a positive test was obtained after incubation in the synthetic solution, organisms were present that grew on nutrient agar.

Two possibilities occurred to the writer. Are the nitrifying organisms filterable or do they get some substance from the bacteria that accompany them, that grow on nutrient agar.

No nitrification was ever obtained from a Berkefeld filtrate of a solution giving a positive test for nitrites or nitrates no matter what was added to the synthetic solution in addition to the inorganic constituents.

The work in connection with the second hypothesis was done only with the nitrite organisms, those oxidizing ammonia to nitrite compounds. In transferring cultures about two c.c. are added to 100 c.c. of the synthetic solution. In a week a strong positive test is obtained, when transfers are again made. If less inoculum is used a longer time is required.

One c.c. of a very weak culture of nitrite bacteria was added to synthetic solutions that had quantities of the following added, Berkefeld filtrate of nutrient agar bacteria, nutrient agar bacteria, dead nutrient agar bacteria, fresh soil, sterilized soil, and Berkfeld filtrate of soil solution, as well as to the check (synthetic solution). It was found after incubation that more nitrite was formed in every case in a given time than in the check. The

fresh soil gave the most brilliant test. Then a series was run as follows: check (synthetic solution), solution plus fresh soil, solution plus sterile soil and solution plus fresh soil. Three-tenths c.c. of nitrite culture was added to the first three. Fresh soil gave best results, sterilized soil not quite as good. Fresh soil wihout the culture gave a slight test after long incubation, showing that the beneficial result was not due to any nitrifying bacteria already in the soil.

It is evident that there is some substance present, in the bacteria that grow on nutrient agar, and in the soil itself, that stimulates these still impure cultures of the "nitrite" bacteria. It was thought that this substance might be in the nature of a vitamin. In order to test this out, to the synthetic solution, small amounts of the following were added: dried autolised yeast, dried yeast, yeast cake, alfalfa and soil. Two sets were run. One set was sterilized and the other not. It was found that all these substances, sterile or not, stimulated the "nitrite" bacteria. Larger amounts of nitrite were formed in all cases, in a given time, than in check. Since the above substances are rich in vitamin A and B, the writer feels there may be a definite relation between food assessory substances and the "nitrite" organisms.

149 (2109)

Some temperature studies on B. acidophilus milk.

By NICHOLAS KOPELOFF AND PHILIP BEERMAN.

[From the Department of Bacteriology, Psychiatric Institute, Ward's Island, New York City.]

Since the beneficial effects of *B. acidophilus* appear to depend upon a transformation of the intestinal flora, it follows that a mass inoculation is desirable. This means a maximum number of viable organisms per c.c. of pabulum. The usual recommendation on commercial preparations of fermented milk is that such milk be kept in a cool place preferably in the ice-box. Consequently, the influence of low temperature on the number of viable *B. acidophilus* in milk seemed worth investigating.

The number of viable *B. acidophilus* in milk held in the ice-box at about 9° C. was determined daily. The viable organisms were killed as follows: after 1 day—about 50 per cent.; after 2 days—about 75 per cent.; after 3 days about 90 per cent., etc. The obvious importance of having a large number of viable organisms is manifested in the necessity for increasing dosage in severe cases. In fact, it is likely that in cases reported as failures, a sufficient increase in the number of viable organisms administered might have resulted in success. The practice of ice-boxing *B. acidophilus* has little to recommend it beyond preserving the palatability of the culture and it is therefore more desirable to keep *B. acidophilus* at room temperature.

A study was made of the influence of time and pressure in autoclaving milk, prior to inoculation with *B. acidophilus*, with respect to the growth of the organism. Milk was sterilized at 15 and 20 pounds pressure in the autoclave for different periods of time and inoculated with *B. acidophilus*. Subcultures were made from each set of flasks for 3 days on milk identically sterilized, thus obtaining acclimatization. On the basis of these comparative tests it was found that milk to be used for inoculation with *B. acidophilus* should be sterilized in the autoclave at 20 pounds pressure for 20 minutes, or at 15 pounds pressure for 20 to 30 minutes. Under these conditions a maximum number of viable organisms is obtained after incubation.

It was necessary for certain experimental purposes to pasteurize *B. acidophilus* milk. By exposing *B. acidophilus* milk to different temperatures for different periods of time it was found that it could be completely sterilized in one litre portions in the Arnold steam sterilizer for 10 minutes (with a final temperature of 81° C.) In attempting to kill *B. acidophilus* in milk by freezing, it was found that after 6 days at about 3° C. the original number of viable organisms was reduced 99.95 per cent. This again emphasizes the drastic action of low temperature on this organism.

150 (2110)

A note on the effects of temperature on the mutant characters "bent" in Drosophila virilis and Drosophila melanogaster.

By C. W. METZ.

[From the Carnegie Institution of Washington, Department of Genetics, Cold Spring Harbor, N. Y.]

The character "bent" in *D. melanogaster* is a member of the "fourth linkage group" in that species.[1] The genes for this and the other two characters now known in this linkage group have been shown by Bridges[2] to be "carried" by the small, dot-like "fourth chromosome." Bent is characterized mainly by two variable modifications, one affecting the wings, the other the legs. The wings vary from "normal" through a series of modified shapes including spread wings, narrow wings, broad wings, swollen wings and most frequently wings that are bent sharply backward at a point near the base. The legs likewise vary from a "normal" condition through a series of stages in which progressive degrees of shortening and twisting is exhibited, beginning with the basal tarsal joint of the hind legs. The extent of the modification seems to be influenced considerably by environmental conditions.

Some time ago I found in *Drosophila virilis* a mutant character which bears considerable resemblance to this bent in *D. melanogaster* both in appearance and behavior. It exhibits much the same series of variable leg and wing modifications, except that those of the wings are less extreme and less frequent—the sharp bend being absent entirely. There are several lines of evidence pointing toward the conclusion that these characters in the two species are homologous, and that the gene for bent is in the small, dot-like chromosome in virilis as it is in melanogaster. Most of this evidence will be considered elsewhere, however. The present paper deals primarily with the effects of temperature on the two characters.

Since the characters show indications of homology and are

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1 Muller, H. J., *J. Exp. Zool.*, 1914, xvii, 325.
2 Bridges, C. B., *Proc. Nat. Ac. Sc.*, 1921, vii, 186.

both influenced by environmental conditions it is of interest to ascertain whether or not they react similarly to similar conditions. The experiments dealing with this problem are still in their preliminary stages, but certain general results have been indicated even by the relatively crude methods used at first. Other agents than temperature have been tried somewhat, but without any definite results thus far.

In the first experiment stock cultures of "bent" virilis were exposed to different degrees of temperature—one at about 9°-12° C., and others at approximately 16°, 23°, and 25° respectively. Each bottle was left at the one temperature during the development of the flies; i. e., up to the time they began to hatch. The three higher temperatures had no observable influence on the bent character, the three lots of flies being essentially like those from ordinary stock bottles of this race. The lot raised in the cold, however, was markedly different. The most conspicuous difference was exhibited by the eyes. In ordinary bent stock of D. virilis the eyes are lightly speckled, due apparently to a disarrangement of the hairs between the facets, and occasionally to a slight disarrangement of the facets themselves. In flies reared in the cold the eyes were decidedly roughened—almost all of the facets being disarranged in some cases. In addition the posterior cross-vein was broken in some individuals, the scutellar bristles were disarranged in many, and other modifications were noted here and there. The wing and leg characteristics, however, showed no exaggeration whatever. In fact they seemed to be less marked than usual.

These results indicated that there was no correlation between the effects on the eyes and those on the legs and wings, and also that cold served to bring out several characteristics of bent that do not appear at ordinary temperatures. They also led, of course, to a similar experiment with the bent race of D. melanogaster. In the latter the eyes are ordinarily normal, not speckled. But when reared in the cold this race also showed the speckling of the eyes. The gene for bent in melanogaster, then, seems to have what may be called the potentiality for speckled eyes like that of virilis, but requires a different environment for producing the effect. In other respects, also, the case parallels the preceding. The legs and wings are, if anything, less extremely affected in the cold than at ordinary temperatures, and some of

the modifications revealed only by the cold in bent virilis are likewise revealed here. These will be noted more specifically below.

Another feature brought out by these experiments is that exposure to cold produces its effect at a fairly definite time in ontogeny. Space forbids going into the details of the experiments, but a few data may be cited. In one experiment a stock bottle (bent virilis) was put directly into the cold when made up and left there for forty seven days at a temperature varying from about 9° to 12° C. This is more than three times the ordinary developmental period in the incubator (23° C.), but the embryos were still in the larval stage when removed from the cold. None of the flies from this bottle showed any noticeable effect of the cold, which suggests that they were not treated at a late enough stage. Another similar experiment gave the same result.

In another experiment a bottle of bent virilis was kept in the incubator as usual for six days, then transferred to the cold for eighteen days, then kept constantly in the incubator. The eyes showed considerable effect in the early counts and then became more like those of ordinary bent in the later ones. In addition other modifications were observed as follows: (1) one or more scutellar bristles misplaced or absent (abbreviation sc); (2) one or more sternopleural bristles absent (stp); (3) apex of fifth vein thickened delta-like (del). Table 1 includes a record of the flies hatching from this bottle, together with the number of days elapsing between the time the bottle was removed from the cold and the time the flies were taken from the bottle. The eye modification, being difficult to classify accurately, is omitted from the table.

TABLE I.

Stock bottle "bent (virilis). Incubator 6 days; cold (approx 9-11°C.) 18 days; incubator remaining time. Pupæ present when removed from cold. The first column indicates the number of days after the bottle was removed from the cold.

Days after cold	del.	del. sc.	del. stp.	del. sc. stp.	sc.	sc. stp.	stp.	none
(7)	8	4	4	2	0	0	0	0
(10)	0	2	0	0	11	0	0	44
(16)	0	0	0	0	0	0	0	57
(17)	0	0	0	0	0	0	0	10
(23)	0	0	0	0	0	0	0	40

It is to be noted that large larvæ were present in the bottle when it was placed in the cold. These were presumably six days old and within twenty-four to forty-eight hours of pupation. When the bottle was removed from the cold a few pupae were present. Presumably these were the first to hatch and are included in the first count; and since all of the flies in this count are modified it may be inferred that these had not passed the critical stage when placed in the cold. This would place the critical stage near the end of the larval period or in the early pupal period. Experiments now under way ought to locate the time accurately. Another noticeable feature in the above experiment is that in the first count all of the eighteen flies are delta-like, whereas in the next count only two out of the thirteen modified flies show this characteristic. This suggests that the effect on the fifth vein is produced relatively later in ontogeny than that on the bristles, particularly the scutellar bristles.

Another bottle carried along with the one just considered, but left in the cold a shorter time gave very similar results. A third treated at a different time and left longer in the cold did likewise, except that only five delta-like flies appeared and these were in the second count instead of the first, although the other modifications appeared in the first.

It has been shown by Krafka[3] that in the bar-eye race of D. *melanogaster* temperature exerts an influence on the extent of reduction of the eye, and that it acts before pupation, during the third to fourth day of development (when the embryo is from 32%-45% developed). Likewise Hoge[4] has shown that temperature affects the manifestation of "reduplicated legs" in the same species. Here it is effective on the egg instead of the larva. The present results more nearly resemble those of Krafka, although the critical period may not come at exactly the same stage in the two cases. They also appear to agree in that the effect is produced before the organs concerned are laid down in the pupa.

The similarity of response to cold on the part of the bent race in D. *melanogaster* was shown by experiments similar to those outlined above. In one of these a lot of bent flies from stock[5]

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[3] *J. Gen. Physiol.*, 1920, v, 433.

[4] *J. Exp. Zool.*, 1915, xviii, 241.

[5] I am indebted to Professor T. H. Morgan for this stock.

was put successively into seven vials, the entire lot being transferred from one vial to the next each time. After being made up the vials were kept in the incubator until after the final transfer, then all were put in the cold for thirty-six days (three times the ordinary developmental period in the incubator). In the first vial the pupae were nearly ready to hatch when placed in the cold and in the others the embryos were successively younger, those in the last being only one day old. From the first three vials many flies hatched while in the cold. From the last none hatched until six days after removal from the cold. No modified flies hatched from the first vial until near the end of the hatch, six days after removal from the cold; and these had normal eyes, (only the bristles affected). In the next vial modified flies appeared earlier and in larger numbers, and so on through the series until in the later vials modified flies appeared in the first counts and were absent from the final counts. The modifications noted were: (1) speckling or roughening of the eyes, (2) abnormal number of arrangement of sterno-pleural bristles, and (3) abnormal number or arrangement of scutellar bristles. The latter was less frequent than in virilis and the former bristle modification usually involved additional bristles instead of fewer as in virilis. Rarely the posterior cross-vein was affected also.

The effects of cold, then, on the bent race of melanogaster agreed with those on bent virilis in that they involved the eyes, the sternopleural bristles and the scutallar bristles. Likewise cold had no effect at all, unless it was an inhibiting effect, on the leg and wing modifications. The only effect of cold found in virilis and not thus far found in melanogaster is the thickening of the apex of the fifth vein. It is also to be noted that the effective period of the cold is localized in both species, although the exact developmental stage at which it comes has not yet been determined.

In conclusion it may be observed that in "normal" stocks of both species reared in the cold none of the above effects has been observed. Also matings of specimens of D. *virilis* showing the scutellar, sternopleural and delta-like modifications, without exposure to cold, have given only ordinary bent offspring. These facts, together with the nature of the results as a whole, are believed to eliminate the possibility of modifying factors or other genetic causes (rather than cold) being primarily responsible. It

seems safe to conclude that the cold simply reveals the "poten-
tialities," so to speak, of the "bent" genes, and that these po-
tentialities are similar in the two species.

151 (2111)

Localization of the vomiting center.

By SOMA WEISS AND ROBERT A. HATCHER.

[*From the Cornell University Medical College, New York City.*]

1. Emesis was induced after destruction of the quadrigemi-
nate bodies, after destruction of the cerebellum, and after sec-
tion of the columns of Goll and Burdach in the cat, and after
destruction of the area described as the vomiting center by
Thumas[1] in the cat and in the dog.

2. Emesis could not be induced by any drug that we em-
ployed after destruction of the sensory nuclei of the vagi in the
cat, nor could it be induced in any of three experiments in this
animal in which the sensory nucleus of only one side had been
destroyed, but vomiting did occur in one experiment in which an
attempt to destroy the sensory nucleus of the right vagus may
have been only partially successful.

3. Results of these experiments indicate that the sensory
nuclei of the vagi are essential for the coordination of the vomit-
ing reflex (that is, for vomiting however induced), and this is in
harmony with our conception of the mechanism of emesis be-
cause: (a) It is well know that the vagus nerve is essential for
emetic action of many drugs. (b) We have been unable to in-
duce vomiting in the cat after destruction of the sensory nuclei
of the vagi while taking especial care to avoid injury to the area
described by Thumas as the vomiting center. (c) There are
no nerve cells concerned so far as known, in the area described
by Thumas.

4. We have shown elsewhere[2] that afferent emetic impulses
from the heart pass by way of the sympathetic nerve, hence the
conclusion is unavoidable that this nerve must make functional
communication with the sensory nuclei of the vagi.

1 Thumas, L. J., *Arch. f. Anat. u. Phys.*, 1891, cxxiii, 44.

2 Hatcher, R. A., and Weiss, Soma, *Arch. Int. Med.*, 1922, vol. xxix, 690.

152 (2112)

Can yeast grow in a chemically pure medium?

By CASIMIR FUNK AND LOUIS FREEDMAN

[*From the Research Laboratory of H. A. Metz, New York City.*]

Reports have lately appeared that yeast can grow on a medium composed of known ingredients, viz.: 50 grams cane sugar (Domino brand), 2 grams KH_2PO_4, 2.35 grams $(NH_4)_2SO_4$, 0.25 gram $CaCl_2$ and 0.25 gram $MgSO_4$, dissolved in a liter of distilled water. It was claimed that on the above medium, which we will call medium O, yeast could be grown in sufficient quantities to serve as a source of vitamine B in animal feeding experiments.

We have attempted to grow yeast on the above medium but have found it difficult to obtain sufficient yeast by this method. It grew very slowly in the incubator and hardly at all at room temperature. The yields were very small; for example, 1800 c.c. of nutritive solution distributed in twelve flasks gave, after growing for a month, 1.5 grams of dried yeast, whereas on addition of 1 c.c. autolyzed yeast to each flask, 900 c.c. in six flasks gave in four days 3 grams of product. Whereas the yeast in the first case developed a brown pigment and presented a spore-like shrunken appearance, in the second case the cells were colorless and in active budding.

In sub-culturing the yeast obtained on medium O, by introducing 5 c.c. into each of a series of flasks containing fresh media, the yield remained almost constant. This excluded the cause of the growth as being due to vitamine D introduced with the seeding, and suggested the possibility of an impurity in the medium. It therefore became necessary to investigate more carefully the purity of one or more of the three constituents of the medium, namely, salts, water and cane sugar.

Each one of the salts used as well as the cane sugar was dissolved separately in distilled water, shaken out with fullers earth and the filtrate evaporated to dryness. The distilled water of the laboratory was redistilled three times in an all-glass apparatus. The salts, sugar and the water were used to make up the neces-

sary solutions, 10 c.c. of each being inoculated with 0.5 c.c. of yeast suspension obtained from the 14th sub-culture. After suitable incubation, the growth was measured in millimeters by the centrifuge method. The results were as follows:

TABLE I.

		Growth in mm. after 3 days	4 days
1.	Medium consisting of purified ingredients (Medium P)	1.5	1.5
2.	Medium P with ordinary sugar replacing purified	5.0	5.0
3.	Medium P with ordinary salts replacing purified	1.5	1.5
4.	Medium P with ordinary water replacing purified	1.5	1.5
5.	Medium O plus one drop autolyzed yeast. Viability test.	25.5	23.5
6.	10 c.c. sterile water plus 0.5 c.c. yeast suspension	1.4	1.5
7.	Same as No. 6, but with yeast killed before incubation	1.0	1.0

These results prove that the cane sugar was the only vitamine D bearing factor in the medium and hence to this factor our whole attention was directed. The cane sugar was recrystallized three times from alcohol and each fraction tested in conjunction with the usual salts and distilled water. The same yeast suspension was used as in the previous experiment.

TABLE II.

		Growth in mm. after 3 days
1.	Medium O	6.12
2.	Medium O with once recryst. sugar replacing ordinary sugar	2.5
3.	Medium O with twice recryst. sugar replacing ordinary sugar	2.37
4.	Medium O with thrice recryst. sugar replacing ordinary sugar	2.12
5.	Medium O plus one drop autolyzed yeast	23.0
6.	Medium P plus ash from one gram of ordinary sugar........	2.12
7.	10 c.c. sterile water plus 0.5 c.c. yeast suspension (killed)	2.0

We have also tested the activity of the residue obtained from the concentrated mother liquors, and found that this concentrate, when purified and added to medium P, influenced the growth of

yeast to a marked degree. This experiment has shown that the impurity in cane sugar can be practically eliminated after one recrystallization from alcohol and that the yeast growth promoting property of cane sugar is not likely to be in the ash. Our work has shown that cane sugar, purified either by fullers earth, by recrystallization from alcohol or by combination of both methods, is devoid of vitamine D.

We have also made some experiments on the influence of the medium on the subsequent growth of yeast. Yeast grown on a medium poor in vitamine D, as medium O, and then used for inoculation, gives a growth amounting to 0.4 mm.; while that grown on an agar-malt medium amounts to 2.25 mm. We can conclude therefore that the yeast which we, at least, have been using is unable to grow on a medium devoid of vitamine D. When growth does take place, it is invariably due to two factors: firstly, the amount of vitamine D introduced with the seeded cells, depending on the type of medium used; and secondly, the vitamine-like impurity found in cane sugar.

This impurity in cane sugar should be taken into account in any subsequent work on this subject. The strain of bakers' yeast that we have been using is unable to grow without vitamine D and hence is unable to synthesize vitamine B in the absence of vitamine D.

153 (2113)

Calcium in the blood.

By WM. C. THRO and MARIE EHN.

[*From the Department of Clinical Pathology, Cornell University Medical College, New York City.*]

In our continued investigation of the calcium in the blood we have paid particular attention to furunculosis and to diabetes. The results here published are part of over 225 determinations made on human beings with the method described by Kramer and Tisdall.[1] They state the normal is 9.2—11.1 mg. in 100 c.c. of blood.

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[1] Kramer, Benj., and Tisdall, F. F., *Jour. of Biol. Chem.*, 1921, xlvii, 475.

In a previous paper[2] we showed that in a few patients with furunculosis the calcium in the blood was low. We can now add to this record the following confirmation of our former results. (The figures represent milligrams in 100 c.c. of blood). 10.8, 10.6, 10.2, 9.9, 8.7, 8.5, (J. H. 7.9), (E. B, 7.1):

In patient E. B. who had had over forty boils and who at the time the blood was taken had a large boil on the arm the calcium content was 7.1. The patient was given 0.1 gram of parathyroid per day and up to the present time an interval of five weeks, there has not been a recurrence. The calcium content was 10.3 after ten days of parathyroid therapy.

In J. H., at the time the blood was taken, there were two acute boils present on the body. After about seven days of parathyroid therapy there is an improvement in the condition.

These findings confirm those of Grove and Vine[3] who record good results from the use of parathyroid in chronic infections.

We have made determinations of the calcium in the blood of children with pneumonia for Dr. J. D. Lyttle and obtained results varying from 5.8 to 10.9. Dr. Lyttle informed us that the amount of calcium so far as he could see had no relation to the prognosis.

Our results from cases of tetany are 8.9, 6.3, 6.4, 11.9, 8.4, 5.6, 7.1, 5.7, 8.1, 7.0, 10.3, 8.4, 12.5, 11.8, 5.6, 7.0. Some of the patients from which these estimates were made also had rickets.

Mendel and Benedict[4] from experiments on animals conclude that if the carbohydrate intake is decreased the calcium excretion is increased, the larger part being eliminated in the feces.

Thayer and Hazen[5] state that in a human subject when the patient was put on a green diet the calcium excretion is increased. Mendel, and Thayer and their associates did not determine the amount of the calcium in the blood.

Below we give the results of the determination of the calcium in the blood of patients in the Physiatric Institute on admission

2 Thro, W. C., and Ehn, M., Proc. Soc. Exp. Biol. and Med., 1921, xviii, 189--191.

3 Grove, W. R., and Vine, H. W. C., Brit. Med. Journ., 1922, I, No. 320, 791; Brit. Med. Journ., 1921, II, 687.

4 Mendel, L. B., and S. R. Benedict, Am. J. Physiol., 1909-10, xxv, 25.

5 Thayer, W. S., and Hazen, Jour. Exp. Med., 1907, ix, 7.

and also after the carbohydrate diet had been decreased.   The
blood was obtained through the kindness of Dr. Sherrill.

DETERMINATIONS OF CALCIUM IN BLOOD OF DIABETES BEFORE
AND AFTER REDUCED CARBOHYDRATE DIET

Patient	Date	Before		Date	After	
		Calcium	Sugar		Calcium	Sugar
McS	3-28-22	5.9	0.367	5-18-22	6.4	0.157
Miss L.	- -22	7.0	0.375	4- 3-22	7.1	0.112
F. R.	3- 1-22	9.0	0.577	5-18-22	5.6	0.129
R.	2-22-22	10.5	0.441	3- 7-22	8.6	0.159
B.	2-16-22	5.7	0.652	2-27-22	6.4	0.341
R.	2-11-22	8.1	--------	3-11-22	8.1	0.166

Calcium determination of the blood of a patient, Mrs. A. G., with osteo-
malacia.

February 8, 1922.   (Had been treated with calcium lactate) 10.1 mg.

February 16, 1922.   9.4 mg.

February 24, 1922.   11.5 mg.

March 1, 1922.   11.6 mg. in uterine blood.

CONCLUSIONS

In some patients with systemic furunculosis, if the blood is
tested at the time an acute boil is present the calcium content is
found to be below normal.

The calcium is below normal in many young children who
have pneumonia.

Not all of the patients with tetany had low calcium.

The placing of diabetic patients on a low carbohydrate diet
did not affect the blood calcium in any definite manner.

One patient with osteomalacia had a normal amount of calcium
in the blood.

154 (2114)

The genesis of gall stones in the dog.

By PEYTON ROUS, P. D. McMASTER, and D. R. DRURY.

[*From the Rockefeller Institute for Medical Research,
New York City.*]

In dogs permanently intubated for the collection of bile, gall
stones not infrequently develop despite the absence of infection,
stasis and gall bladder activity.   The character of the stones has
already been discussed.[1]   They are always discrete to begin with,

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1 Rous, Peyton and McMaster, P. D., *Jour. Exper. Med.*, 1921, xxxiv, 47.

scattered upon the glass and rubber wall of the collecting tube. What determines this punctate localization? In certain instances, of almost pure calcium carbonate concretions, the answer is plain. These form in the midst of organic debris, not infrequently around bits of talc from the tube surface. In other cases minute, rounded, pigmented particles from the bile lodge upon the tube wall, and stone formation takes place upon these as nuclei. To trace the source of such particles and their significance we have made day to day studies of the sediment from sterile 24 hour specimens of bile centrifuged on removal from collecting balloons devoid of air. Also we have followed the early stages of calculus formation in the collecting tubes of the same animals.

The bile was found to yield formed elements identical with those later recovered from the tube system and from the interior of stones forming upon the walls of the latter. The nature and the amount of the sediment vary with the condition of the animal. For a day after the operation whereby intubation is effected it may consist merely of mucus, which later is seldom met with. Usually one obtains from the specimen of the second 24 hours after operation and perhaps more abundantly from that of the third, a slight brown deposit, made up, as the microscope shows, of minute, highly refractile, translucent, yellow-brown granules. The shape of these tends to be spherical, but is rendered various by the partial merging of the spheres. They fracture radially on pressure into rosettes, are anisotropic, and fail to lose this character or their shape when heated to 100° C. The refractile material of which they are mainly composed is insoluble in water, alcohol, ether, chloroform, or acetone, but dissolves readily in chloroform after treatment with acid, as also in a dilute watery solution of hydrochloric acid, leaving the brown pigment and a mucous "shadow" behind. They are colored deep blue with Nile blue sulphate but are unaffected by the stains for neutral fats, and are Gram-negative. Such granules serve as nuclei for the deposition of calcium carbonate and calcium bilirubinate, as further study of the bile has shown. In the secretion of the third to fifth day after operation, but usually not later, they are present but encrusted with a deposit of more or less pigmented crystals composed of a mixture of the salts mentioned. Thereafter this crystalline matter alone is to be found, unless there occurs some liver disturbance, when a shower of the brown granules

may again appear in the bile and crystalline deposition again takes place upon them. We have observed such a sequence of events after poisoning with chlorform or toluylenediamine, after biliary obstruction of some days' duration, and following intravenous injection of a concentrated solution of calcium chloride. The majority of the stones forming upon the canula and collecting tube within the animal have such granules as their nuclei.

Whence come these granules? They suggest in size and shape the "bile thrombi" observed under pathological conditions within liver tissue. By digesting the tissue with trypsin such "thrombi" can be obtained separately, and their characters compared with those of the granules,—from which they are then found to differ in many respects, notably in being isotropic. Yet our observations leave no doubt that "thrombi" identical with those in the liver do sometimes appear in the bile. Whether they have any relation to gall stones has not been determined.

The dogs studied were losing all of their bile. May this not have been a factor in the cholelithiasis? That it was not a primary factor was shown by interpolating small glass tubes into duct systems left with intestinal connection undisturbed. In some of the animals thus treated, stones of the characteristic sort formed upon the glass. This being true, why were they never found in the ducts, or, more especially, in the gall bladder of healthy animals? Foreign bodies left in the latter viscus under aseptic conditions fail to bring about the development of stones.[2] The ducts, elaborating, as they do, a secretion of their own,[3] and provided with a musculature, may well be able to rid themselves of particulate matter. But the shortcomings in this connection of the gall bladder are strikingly proven by the frequent occurrence in it of a shreddy, cellular debris which, if present in a glass-rubber system, would almost infallibly lead to a formation of calculi. The cause for the absence of stones from the gall bladder is to be found in the change in the reaction of the bile which this organ effects. It acts to render the secretion acid, even acid to litmus, as others have noted before us.[4] The P_H of hepatic duct bile, as determined electrometrically, ranges between 7.5 and

[2] Mignot, R., *Arch. gen. de Med.*, 1898, I, VIII Ser., T. X., 129.

[3] Rous, Peyton and McMaster, P. D., *Jour. Exper. Med.*, 1921, xxxiv, 75.

[4] Okada, S., *Jour. Phys.*, 1915-16, 1, 114; Neilson, N. M., and Meyer, K. F., *Jour. Infect. Dis.*, 1921, xxviii, 130.

8.5, while that of gall-bladder bile from the same animal may fall as low as 5.18. Test tube experiments show that when liver bile containing a crystalline sediment of the sort out of which stones form is gradually brought to the average reaction of bladder bile, by the addition of N/10 HCl, or 3 per cent. acetic acid or concentrated lactic acid, the sediment goes into solution.

From these observations the inference seems justified that many sorts of liver derangement conduce to the presence, in bile that is sterile, of potential nuclei for calculus formation. But the normal gall bladder effects precisely the sort of change in the fluid that renders stone formation upon such nuclei impossible. The organ does not merely concentrate[5] and store the bile, it alters it so that it can be stored safely. In the lack of such alteration, as when the gall bladder fails to function normally, it is not surprising that stones should form.

There are facts in the literature which indicate that the principles here set forth apply to other species besides the dog. The reaction of rabbit bile from gall bladders rendered abnormal by typhoid infection is not acid like that from the healthy organ but has the same alkalinity as liver bile.[4] And in such gall bladders stones composed of calcium salts are regularly found if the animal lives long.[6] This cholelithiasis has heretofore been attributed to infection and inflammation, factors which are, at the most, accessory judging from the evidence here presented. We are now attempting to determine whether the change in reaction has any relation to the state of the cholesterin in human bile.

5 Rous, Peyton, and McMaster, P. D., *Jour. Exp. Med.*, 1921, xxxiv, 47.

6 Meyer, K. F., Neilson, N. M., and Feusier, M. L., *Jour. Infect. Dis*, 1921, xxviii, 76.

155 (2115)

The cause of low plasma protein concentration in nephritis.

By G. C. LINDER, C. LUNDSGAARD, D. D. VAN SLYKE,
and E. STILLMAN.

[*From the Hospital of The Rockefeller Institute for Medical
Research, New York City.*]

The reduction in concentration of plasma proteins observed in a considerable proportion of nephritic patients has been attributed by some authors to a decrease in the amount of plasma proteins in the body, by others to a dilution of the blood with water (hydremic plethora). We have made one or more determinations of the blood volume by Keith, Rowntree, and Geraghty's "Vital Red" method on all but three of the patients above reported with reduced plasma protein concentration, and on some others.

The cause of the reduced protein concentration was found to be not hydremic plethora, but an actual decrease in the amount of plasma proteins in the body. Normal blood volumes were found, even in cases of extreme edema. The amount of total plasma proteins per kilo body weight (the weight being corrected as nearly as possible for estimated edema fluid present) however, varied from 1.5 to 3.0 grams, compared with 3.5 grams found in normal individuals.

156 (2116)

The globulin and albumin content of the plasma in nephritis.

By G. C. LINDER, C. LUNDSGAARD, and D. D. VAN SLYKE.

[From the Hospital of The Rockefeller Institute for Medical Research, New York City.]

Bright and a number of subsequent observers have described a diminution of proteins in the plasma of some persons suffering from albuminuria and edema; and later it has also been found that the decrease occurs chiefly in the plasma albumin (Epstein) the globulin not being diminished.

We have determined the albumin and globulin over varying periods of time in the plasmas of a number of nephritics by the recent method of Howe, with the results indicated below. The cases were classified as glomerular nephritis, nephrosis, and nephrosclerosis according to Volhard and Fahr.

	Type of Nephritis.			
	Glomerular nephritis.	Nephrosis.	Nephro-sclerosis.	Functional albuminuria
Number of cases........	13	2	3	2
Cases with low total plasma protein (3.5 to 5.5 per cent.)......	10	1	0	0
Cases with normal plasma protein (6 to 7 per cent.).........	3	1	3	2
Cases with low albumin/globulin ratio (below 1.4)...............	12	2	0	1

In glomerular nephritis a return to a normal total protein content has been observed in some cases, but a return to a normal albumin: globulin ratio has not yet been observed in any of our cases.

In one typical nephrosis patient a great loss of edema was observed without any change in plasma proteins, but *after* the edema had disappeared the total proteins began to rise towards the normal. The case of nephrosis with normal total content at the first examination was already convalescent.

157 (2117)

The demonstration of a hormone in plant tissues to be known as "glucokinin."

By J. B. COLLIP.

[From the Department of Biochemistry of the University of Alberta, Alberta, Canada.]

The demonstration by the writer, in collaboration with Mac-Leod, Banting, and Best, that active preparations of the internal secretion of the pancreas conferred upon the depancreatized dog the power of glycogen formation led at once to the idea that wherever glycogen occurred in nature, a hormone similar to that produced by the islet cells of the pancreas would probably be found. Three obvious places to look for such a substance were[1] tissues of lower animals rich in glycogen such as the clam,[2] yeast,[3] fungi. The writer[2] was immediately successful in demonstrating the presence of such a hormone in clam tissue. Yeast was also investigated continuously for many months, and on January 26, 1923, after more than a score of failures, an extract of yeast was obtained which produced marked hypoglycemia in a normal rabbit (blood sugar 0.046 per cent). Since that date extracts of yeasts which have similar properties have been prepared by five different methods. The administration of such a potent yeast extract to a depancreatized dog also caused a marked fall in the percentage amount of blood sugar and a great decrease in the hourly excretion of sugar. As the yeast organism is a plant of the least differentiated type in the vegetable kingdom, the idea occurred to the writer that, as all plants are sugar burners as well as producers, the preparation of the sugar molecule for combustion in the protoplasmic fire of the plant cell might be quite a secondary affair and be dependent, as Winter and Smith[3] have suggested, on the preliminary formation of γ glucose, the combustion of sugar or the polymerization of the same being primarily dependent upon presence of the γ form of glucose. If this

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1 Banting, F. G., Best, C. H., Collip, J. B., MacLeod, J. R. R., and Noble, E. C., *Trans. Roy. Soc. Can.* 1922, xvi.

2 Collip, J. B., *J. Biol. Chem.*, 1923, lv. XXXIX.

3 Winter, L. B., and Smith, W., *J. Physiol.*, 1922, lvii, 100.

theory were correct one should be able to demonstrate the hormone in plant tissue which contained neither glycogen nor starch. Professor F. J. Lewis very kindly suggested the onion as a type plant which contains neither glycogen nor starch, and which is also a well recognized glucose burner. The onion was therefore used and the writer was successful in preparing extracts from onion tissue which produced when administered to normal rabbits effects similar to those following the administration of yeast extracts. A depancreatized dog was caused to have a normal blood sugar for a period of many hours by the use of onion extract, and the urine was rendered practically sugar free for a similar period.

Encouraged by these results, the writer investigated tissues of other plants and similar results were obtained in many instances. Extracts made from yeast (either baker's or brewer's), green onion tops, onion roots, barley roots, sprouted grain, green wheat leaves, bean tops, and lettuce have been found to have similar properties.

The demonstration that a substance capable of producing hypoglycemia in normal rabbits and in the few cases tested out, a definite fall in the blood sugar and a decrease in sugar excretion of depancreatized dogs by extracts of plant tissues so widely divergent in character as the above list indicates, justifies one in assuming a hormone present in the above plant tissues and probably universally present in plant tissue. Such hormone would be just as essential to the metabolism of sugar in the plant as a similar hormone, produced in the higher animal by the islets of Langerhans, is to the metabolism of sugar in the animal.

The new substance, although in some ways similar in its properties to the active principle of the pancreas of animals, obviously can not be known as "Insulin." In the official announcement[4] by the Toronto group on the effects of extracts of pancreas on diabetes, the name "Insulin" was given to an extract of animal pancreas prepared by a definite process elaborated by the writer and known as the "Collip method." Therefore it would seem proper to suggest that this new hormone derived from plant sources be called "Glucokinin."

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[4] Banting, F. G., Best, C. H., Collip, J. B., Campbell, W. R., Fletcher, A. A., Macleod, J. J. R., and Noble, E. C., *Trans. Assoc. Am. Physicians*, May, 1922.

That this hormone will be useful in the treatment of diabetes mellitus in the human subject there can be little doubt. Judging by the results obtained on diabetic animals it will in some ways be much superior to "Insulin." Its effect develops slowly and is long maintained. The fact that relatively crude extracts of many plant tissues are practically non-toxic is also a factor of great practical importance. A few results are indicated in Table 1.

TABLE 1.

Animal.	Source of extract.	Blood sugar.	
		Control.	Low point.
Normal rabbit	Yeast	0.110	0.046
Normal rabbit	Yeast	0.118	0.046
Normal rabbit	Yeast	0.080	0.038
Normal rabbit	Onion	0.118	0.058
Normal rabbit	Lettuce	0.094	0.056
Normal rabbit	Wheat leaves	0.103	0.065
Normal rabbit	Wheat leaves	0.106	0.058
Normal rabbit	Bean greens.	0.095	0.065
Depancreatized dog...........	Onion	0.190	0.090

158 (2118)

Evidence of the dynamic importance of auricular systole in man.

By HAROLD S. FEIL and LOUIS N. KATZ.

[*From the Medical Clinic of Western Reserve University at City Hospital, Cleveland, Ohio.*]

Considerable discussion has arisen as to the dynamic importance of auricular systole. Some of the differences of opinion are no doubt due to the fact that the vigor of auricular contraction varies considerably under different experimental conditions. The idea has occurred to many that evidence of the dynamic importance of auricular systole might be obtained *in man* by comparing the ventricular efficiency during normal action with that found in auricular fibrillation. But nothing has come of this suggestion because no adequate criterion for comparing the ventricular efficiency had been found. In this report we wish to suggest that an accurate study of the duration of total ventricu-

lar systole and the phase of systolic ejection may offer a criterion of ventricular efficiency as determined by auricular systole.

Our attention was directed to this question in the course of an investigation into the duration of ventricular systole in fibrillation of the auricles. Inasmuch as the venous pressures are considerably increased in this condition, we anticipated, on the basis of animal experiments, that the duration of systole and its ejection phase would be greater than in corresponding cycles of normal hearts. Contrary to expectations, however, we found that on the whole the length of total systole as well as the duration of the ejection phase was definitely shorter than normal. This shortening was apparently unrelated to the age, blood pressure, medication or etiology of the fibrillation.

In attempting to explain this we were fortunate in finding a patient in whom periods of auricular fibrillation alternated spontaneously with a normal mechanism, as established by electrocardiograms. Fortunate also was the fact that the heart rate remained rapid during the periods of normal mechanism. This permitted calculations at approximately the same heart rates.

Results: The measurements of twenty-two beats during fibrillation of the auricles and of a like number during normal heart action gave the figures shown in Table 1. They indicate that

TABLE 1.

Cardiac mechanism	Predominant heart rate (beats per min.)	Duration of preceding diastole (average)	Duration of total ventricular systole (average)	Duration of systolic ejection phase (average)	Duration of "systole", as calculated from formula $S = .31\sqrt{C}$
Auricular fibrillation	151	0.222 sec.	0.175 sec.	0.128 sec.	0.195 sec.
Normal mechanism	130	0.234 sec.	0.226 sec.	0.171 sec.	0.210 sec.

under otherwise practically constant conditions the duration of systolic ejection and total systole were shorter during the period of auricular fibrillation than during the interval in which normal auricular contractions were present.

These effects can be interpreted in the following way—indeed, as previously indicated, they can not be explained in any other manner. During the synergic auricular contractions which occur

normally, an additional ventricular filling takes place or at least the intra-ventricular pressure is increased. This operates to lengthen the duration of the systolic ejection phase and through this the length of the entire systole. When the synergic auricular contractions are in abeyance as in auricular fibrillation, the ventricles either fill less efficiently or at least the initial tension is not as high, in spite of a considerable venous pressure in the veins.

Summary: 1. The duration of ventricular systole and its ejection phase are influenced at corresponding heart rates by the diastolic filling and the initial pressure of the ventricles, as demonstrated by recent experimental work.

2. The duration of these periods in hearts with auricular fibrillation is shorter than normal at corresponding heart rates, even in spite of the higher venous pressures present.

3. These intervals increase when the heart reverts to a normal mechanism.

We conclude, therefore, that these observations give probable if indirect evidence of the dynamic importance of auricular systole in the normal heart beat in man.

159 (2119)

Kahn precipitation test for syphilis—improved procedure.

By R. L. KAHN.

[*From the Bureau of Laboratories, Michigan Department of Health, Lansing, Michigan.*]

The precipitation test for syphilis proposed by the author[1] called forth favorable comment from a number of investigators (Keim and Wile,[2] Herrold,[3] Young,[4] Ide and Smith,[5] Holmes[6]

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1 Proc. Soc. Exp. Biol. and Med., 1922, xix, 182; *Arch. Derm. and Syphil.*, 1922, v, 570 and 734, vi, 332.

2 *J. Amer. Med. Ass'n.*, 1922, lxxix, 870.

3 *J. Amer. Med. Ass'n*, 1922, lxxix, 957.

4 *J. Amer. Med. Ass'n*, 1922, lxxix, 1674; *Amer. J. of Public Health*, 1923, xiii, 96.

5 *Arch. Derm. and Syphil.*, 1922, vi, 770.

6 *J. Mo. State Med. Ass'n*, 1922, xix, 479.

and Levin[7]).   Since the publication of the preliminary studies, however, several observations have been made which led to some changes in the original procedure; also, a modified technic has been evolved which is employed as a check on the original one. The improved method discussed in the following resumé embraces both the original and modified procedures.

THE ANTIGEN

Preparation of Alcoholic Extract: Beef heart is freed from fat and fiber in the usual manner and passed several times through a meat grinder.   It is then spread on a platter and dried by means of a revolving fan.   The dried plates are broken up into small particles and ground in a mortar or coffee grinder.   The ground muscle is then extracted with ether at ice-box temperature until supernatant ether is free from coloring matter.   Between three and four ether extractions will bring this about.   At the end of the final extraction, the ether is filtered off and the ground muscle dried for some hours at room temperature until free from ether odor.

Given quantities of dried material are placed in Erlenmeyer flasks.   A quantity of 95 per cent. alcohol equivalent to five times the amount of dried material is added to each flask.   The extraction is carried out in the ice-box from nine to ten days.   After that period, 10 c.c. of supernatant extract is pipetted into a large test tube and the color compared either with some antigen that is known to give good results or with the following approximate color standard:

1. A solution is prepared containing 0.5 gm. potassium bichromate ($K_2Cr_2O_7$) in 100 c.c. distilled water (permanent standard solution).

2. One c.c. of this solution is mixed with 75 c.c. distilled water.

3. Ten c.c. of solution 2 is measured into a tube of the same size containing the extract to be tested and the colors compared.

If the amount of coloring matter in the new extract is weaker than in the standard, room temperature or incubator temperature for some hours or overnight may be resorted to until coloring matter is brought up to that contained in the standard.   If the

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[7] *J. Kans. State Med. Ass'n*, 1923, xxiii, 4.

color of new extract is the same or more intense than the standard, the extraction may be considered completed. The extract is now filtered off and is kept in the dark at room temperature as stock solution. This extract will keep for at least a year and possibly for many years.

Antigen prepared from ground heart muscle kept for some weeks or months does not give as sensitive results as that prepared from material freshly ground and dried. It has furthermore been observed that the non-specific sediments in negative sera following incubation of serum and antigen are due to impurities in the extract. These impurities are caused, in most cases, by excessive contact of the extract with cork or rubber stoppers.

The use of an alcoholic non-cholesterinized antigen for this test has been discussed in previous studies. Further investigations are under way. At present, in routine work, the use of a cholesterinized antigen only is recommended and the procedures discussed below apply to this type of antigen.

Cholesterinization of Antigen: A given amount of extract is measured into an Erlenmeyer flask and a quantity of cholesterin added to render it a 0.4 per cent. solution. The cholesterin is dissolved by warming in a water bath with gentle rotation. The solution is then filtered to remove impurities and is ready for use.

We have recently observed that different lots of alcoholic extract are capable of holding in solution different amounts of cholesterin. Thus, one extract appears to be saturated on adding 400 mgm. of cholesterin per 100 c.c., whereas another extract is capable of holding as much as 600 mgm. in solution, and even more. The probable explanation for this is the varying lots of extract, although possessing approximately the same color range, may contain different amounts of lipoid. One may then expect that an extract comparatively poor in lipoids will be capable of dissolving more cholesterin before reaching the saturation point than one which is rich in lipoids. This suggested a method for standardizing the lipoid content of antigens for this test aside from the approximate standardization of these antigens by means of a color range discussed above. The method consists in adding sufficient cholesterin to given alcoholic extracts to bring them to the saturation point at a given temperature.

An alcoholic extract is capable of holding in solution considerably more cholesterin at incubator than at room temperature.

If we choose the latter temperature as our standard, we may find that extract A becomes saturated on adding to it 400 mgm. of cholsterin per 100 c.c.; extract B reaches saturation with 500 mgm., and extract C is capable of holding in solution as much as 600 mgm. of cholesterin per 100 c.c. before reaching the saturation point. These three cholesterinized extracts according to our preliminary experiments, approach one another in sensitiveness. Considerably more work will have to be done in this connection. We may find that to replace the lack of non-cholesterin lipoids in a given extract with cholesterin, may lead to difficulties. A number of extracts prepared from different heart muscles are now being tried out and the results with detailed method of standardization of antigen will be reported in a forthcoming paper. It is touched upon here in order to indicate to workers interested in this test, a clue by which one may overcome the variable elements which enter in the alcoholic extraction of different lots of beef hearts.

At present we recommend standardizing the alcoholic extract by means of an approximate color standard as indicated above and cholesterinizing it by adding 400 mgm. per 100 c.c. This amount of cholesterin approaches the saturation point at room temperature of most extracts and is giving good results in our routine work in this laboratory and, to our knowledge, in other laboratories where this test is employed as a regular procedure.

It is well to cholesterinize amounts of extract which will be likely to last for about a month or two only. Such extracts show a tendency to become slightly less sensitive on prolonged standing.

If a given extract is incapable of holding in solution 400 mgm. of cholesterin per 100 c.c. at room temperature, the mixture should be kept in the incubator in the dark and the tendency of the crystalization of the cholesterin will thus be avoided.

PROCEDURE I (ORIGINAL).

Principle: The mechanism of this procedure is believed to be as follows. The antigen consists of a highly concentrated solution of apparently specific lipoids. Approximately the smallest amount of salt solution (0.85 per cent. NaCl) is added to a given amount of antigen which will result in an opalescent mixture. This renders the mixture unstable with reference to precipitation as indicated by the fact that it will, in practically all cases, become

turbid on standing or in the cold. Since the mode of adding salt solution to antigen markedly affects the final mixture, a method is indicated for this purpose in which tubes of a given calibre are employed; the aim being to produce mixtures which, although clear, are on the verge of precipitation. The employment of a minimum amount of salt solution furthermore eliminates the inhibitory effect of excessive amounts of this solution on specific precipitation of serum and antigen. Assuming therefore that we are dealing with an unstable antigen salt-solution mixture and assuming further that the reacting substances of syphilitic serum are probably also relatively unstable, as indicated by the quantitative fluctuations of these substances during the course of the disease, one might expect that mixing these two unstable substances would result in precipitation.

Dilution of Antigen for Tests: The following method of diluting the antigen with salt solution is somewhat simpler than that described in the earlier communications:

1. The amount of antigen required for the tests is measured into an agglutination tube of about 0.8 cm. diameter.

2. Three times the amount of salt solution is added to a similar tube.

3. The saline is poured into the antigen tube with reasonable rapidity and the mixture is immediately poured back into the original antigen tube.

4. This mixture, which is opalescent and shows no signs of turbidity, is now ready for use, although there is no harm in further mixing back and forth.

The Test: Three-tenths c.c. of serum, previously inactivated for one-half hour at 56° C., is measured into a small tube and 0.05 c.c. of antigen-salt solution mixture is added to it, and shaken for about a minute or more. Known positive and negative sera form the controls. The tubes are observed for spontaneous reactions and the final results are read after overnight incubation at 37° C. Best results are obtained with sera that are clear and to which sheep cells (for removal of natural amboceptor) have not been added.

Increasing Sensitiveness of Test: If an antigen, after testing with a number of syphilitic sera, appears to lack sensitiveness, the following simple steps will help overcome this difficulty:

1. The salt solution is chilled by keeping it in the ice-box before

mixing with antigen. This renders the final antigen-salt solution mixture somewhat less stable than mixtures prepared with salt solution kept at room temperature. 2. Instead of mixing 3 parts of salt solution with 1 part of antigen, 2.5 parts of salt solution are mixed with 1 part of antigen. This increases the instability of the final mixture with reference to precipitation. We have not had occasion to use less than 2.5 parts of salt solution with 1 part of antigen in our work.

The important thing to keep in mind is that the antigen-salt solution mixture used in the tests must show no signs of turbidity. An antigen mixture showing even slight turbidity will be likely to give false weak reactions.

Procedure II (Modified)

Principle: The mechanism of this procedure is believed to be somewhat different from that of procedure I. Antigen and salt solution are mixed in such proportions that the major part of the lipoids is precipitated in a very fine state. The precipitate is obtained free from other elements by centrifugation. On resuspending this precipitate in salt solution, there results a milky but opalescent mixture. This mixture shows no trace of visible precipitate but undoubtedly consists of a suspension of lipoid particles in a very fine form, and when mixed with serum is apparently capable of combining readily with the specific elements of the latter. (The application of this principle to other antigens will be discussed in forthcoming studies).

Dilution of Antigen for Tests:

1. A given amount of antigen (depending on number of tests) is measured into a small tube and an equal amount of salt solution is added to it either from a pipette or from another tube.

2. This is mixed and centrifuged for about five minutes— until the supernatant fluid is practically clear and a white precipitate is settled on the bottom of the tube.

3. The supernatant fluid is poured off and discarded and the amount replaced with salt solution. (*Ex.*: 0.5 c.c. antigen is mixed with 0.5 c.c. saline and centrifuged. Supernatant fluid is poured off and 1 c.c. saline added).

4. On mixing, the precipitate is redissolved in the salt solution,

forming a milky, opalescent mixture with no trace of a precipitate. This mixture is then ready for use.

The Test: Three-tenths c.c. of serum, previously inactivated for one-half hour at 56° C., is measured into a small tube and 0.05 c.c. of new antigen mixture added to it. The tube is shaken for about a minute. Practically all tubes will become slightly cloudy but those showing spontaneous reactions will show definite precipitations in clear serums. The final reading in this case also is taken after overnight incubation at 37° C.

THE READING OF RESULTS

The results are read in accordance with the following scale:

1. One or more large clumps = ++++.
2. Large sized flocculi = +++.
3. Moderate sized flocculi or granules = ++.
4. Small flocculi or granules = +.
5. Very small flocculi or granules = ±.

It is recommended in reading the results that all tubes showing the presence of definite clumps or heavy precipitates in both procedures be first picked out and set aside in a special rack. These are the definitely positive reactions (++++ and +++) and can be read with very little difficulty. The remaining tests carried out with Procedure I are read as follows: 1. Slant the tube to such an extent that it is almost horizontal. This causes the fluid to spread into a thin layer. 2. Hold the slanted tube some inches above the level of the eyes. 3. Focus against some dark object such as the lower part of a window shade. 4. Observe whether the thin layer of fluid is entirely clear or has fine particles evenly distributed.

All remaining tubes of Procedure II receive one c.c. of salt solution each and rack is gently shaken and permitted to stand about ten minutes. The negative tests show opalescence while the positive tests show the presence of precipitates. With some antigens this procedure is unusually sensitive and doubtful (±) reactions may safely be considered negative. Salt solution may occasionally be used with advantage also in diluting some tests carried out with Procedure I.

The results of the two methods outlined check very closely. In isolated cases where there is disagreement, the average finding of the two methods is taken as the final result.

Although the final results are read after overnight incubation, it will be found that the strongly positive serums either react spontaneously after adding antigen or show the presence of definite precipitates after several hours incubation. From fifteen to seventeen hours is more than ample for incubation. Prolonged incubation beyond these hours is to be avoided. An element which will give false weak reactions particularly after prolonged incubation is the employment of tubes which will permit considerable evaporation of the serum during the incubation period. Agglutination tubes having an inner diameter of about 0.8 cm. will be found to give best results.

We have not found it necessary to employ sterile salt solution. Chemically clean but not sterile precautions are required in this test.

STATUS OF IMPROVED PROCEDURE.

The combination of the two steps outlined, together with proper negative and positive controls, forms in our experience a more dependable test than that originally described.

160 (2120)

Dilution of antigen for Wassermann test.

By R. L. KAHN.

[From the Bureau of Laboratories, Michigan Department of Health, Lansing, Michigan.]

In the previous communication, the author described a method for preparing antigen for the Kahn Precipitation Test and showed (Procedure II) that the sediment formed on mixing and centrifuging equal quantities of antigen and salt solution may be redissolved in salt solution and employed in the above test for syphilis. The question came up whether the same sediment taken up in salt solution may not be used as an antigen in the Wassermann test and the following experiments were carried out accordingly.

A cholesterinized extract regularly used in the Wasserman test in a dilution of 1 : 70 with salt solution was employed. One c.c. of this extract was measured into a small tube and one c.c. of salt solution added to it. This was mixed and centrifuged, after which the supernatant fluid was poured off and the sediment suspended in 70 c.c. salt solution. This mixture was tested for anti-complementary, hæmolytic and antigenic properties side by side with a suspension prepared by slowly adding 70 c.c. saline to 1 c.c. antigen.

It was found that the suspension prepared from the sediment which resulted from mixing equal amounts of salt solution and antigen was considerably less anticomplementary as well as less hæmolytic than the suspension prepared by adding salt solution to antigen in the regular manner. It was further found that the antigenic properties of the suspensions resulting from either of the two modes of mixing with salt solution was about the same. Similar experiments gave the same results.

Clinical studies may ultimately establish that salt solution suspensions of the lipoid sediment possess high specificity and that the occasional non-specific Wassermann reaction given by cholesterinized antigens may be avoided by employing resuspended antigen-salt solution sediments.

161 (2121)

A phyto-pharmacological study of some heart drugs.

By DAVID I. MACHT and DOROTHY S. LUBIN.

[*From the Pharmacological Laboratory, Johns Hopkins University, Baltimore, Maryland.*]

A number of heart drugs or poisons belonging to the digitalis group were examined in respect to their toxicity for plant protoplasm. The method used was the same as that followed by the authors in a study of cocaine, alcohols, quinine alkaloids, etc.[1]

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[1] Macht, D. I., and M. Livingston, *J. Gen. Physiol.*, 1922, iv, 573.

The effect of the drugs was studied on the growth or elongation of the hypocotyls of three-day-old seedings of *lupinus albus*. The following drugs were examined, digitoxin, digitalin (German, Merck), digitalin Kiliani, digitonin, strophanthin U. S. P., ouabain or crystalline strophanthin and bufagin. Those principles which were not soluble in water were used in a 1 per cent. solution of ethyl alcohol, the control in such cases containing also the same amount of alcohol.

It was found that most of the above bodies were not very toxic for the plants, with the exception of bufagin. Thus digitoxin 1:100,000 gave a growth of 87 per cent. as compared with the control plants in normal Shive solution. Digitalin, Merck in the same concentration gave a growth of 86 per cent. Digitalin Kiliani in concentration of 1:500,000 gave a growth of 49 per cent., while solutions from 1:100,000 gave a growth of 98 per cent. Digitonin solution 1:100,000 gave a growth of 82 per cent. Strophanthin U. S. P. 1:100,000 gave a growth of 73 per cent. and ouabain in the same concentration gave a growth of 85 per cent.

The effects of bufagin were very different. Whereas Abel and Macht[2] found this to be an extremely potent drug for animals, its toxicity for the heart being compared to that of digitoxin and strophanthin, it was found to be very little toxic for plant protoplasm. Thus, while ouabin exerted practically no inhibition on the growth of the lupine (97 per cent) and strophanthin U. S. P. in concentrations of 1:100,00 gave 73 per cent. growth and 1:50,000 gave 63 per cent. of growth, it was found that bufagin in solutions 1:100,000 gave only 20 per cent. of growth and in solutions of 1:50,000 gave only 15 per cent. of growth as compared with the normal controls. This difference in the toxicity for plants between bufagin and the strophanthins and the other heart drugs studied is of special interest in the first place because bufagin from a zoopharmacological and chemical standpoint is classed with the digitalis principles and in the second place because that poison is of animal origin. This agrees with the many other instances found by the authors of the *much greater toxicity of many poisons of animal origin as compared with poisons of plant origin for plant protoplasm.*

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2 Abel, J. J., and D. I. Macht, *J. Pharm. and Exper. Therap.*, 1910-11, iii, 319.

The authors have also made a study of some galenical prepara-
tions and more particularly of tincture of digitalis in respect to
their toxicity for lupinus albus. It was found that various dilu-
tions of tincture of digitalis containing the same amount of alco-
hol were progressively less toxic for the lupinus so that a curve
could be plotted expressing the relationship between the toxicity
of the various concentrations and the growth of the plant, run-
ning more or less parallel to a similar curve obtained for the
toxicity of digitalis by the cat method.

162 (2122)

An effect of x-rays on crossingover in drosophila.

By JAMES W. MAVOR.

[*From the Department of Biology, Union College, Schenectady,
New York.*]

In Drosophila melanogaster when a white-eyed, long-winged
female is crossed with an eosin-eyed, miniature-winged male,
the daughters are all heterozygous and may be represented by
the formula $\dfrac{w \quad M}{W^e \quad m}$, indicating that one of the X chromosomes
carries the determiners for white eye-color (w) and long wings
(M) while the other carries the determiners for eosin eye-color
(W^e) and miniature wings (m). If such a heterozygous female
is bred she will have four kinds of regular sons irrespective of
the male with which she is crossed, since the regular sons obtain
their X chromosomes from their mother only. In two of the
kinds of sons the characters will appear as they entered in the
original cross, *i. e.,* one kind will be white-eyed and long-winged
and the other eosin-eyed and miniature-winged; these make up
the noncrossover classes. In the other two kinds of sons the
characters will be interchanged, *i. e.,* one kind will be white-eyed
and miniature-winged and the other eosin-eyed and long-winged;
these make up the crossover classes. It is usual in work on

crossingover to cross the heterozygous females with a recessive male, which in this case would be white-eyed and miniature-winged, in order that the daughters may also show the crossover and noncrossover classes. The experiments to be described were designed primarily to test for nondisjunction and for that reason the females were mated to males with the dominant character red-eyed so that the exceptional sons and daughters could be recognized. This did not of course affect the character of the regular sons and it is from counts of these that the crossover values have been determined. No attempt has been made to correct the data for double crossingover.

The first series of experiments in which the effect of X-rays on crossingover was tested were those of our third series of X-ray experiments. Each group of females was treated only once but the X-ray dose was different in the different groups. The females were mated immediately after the treatment to wild-type males. The X-rayed and control females were allowed to remain in the first bottles for six days and in the second bottles for eight days. The F_1 were counted in the bottles until eighteen days after the parents were placed in the bottles, the temperature of the incubator being kept between 73° and 76° F. In the first bottles there was no significant difference between the crossover values of the control and X-rayed females. On the other hand in the second bottles there was a significant decrease in the crossover value of the X-rayed females whenever a sufficient number of F_1 were obtained to give a significant result. The totals for all the experiments of the series show a difference between the control and X-rayed females of 14.97 times the probable error and a difference of 9.31 times the probable error between the first and second bottles of the X-rayed females. The experiments also show that the crossover value decreases as the X-ray dose increases.

In the fourth series of experiments, the second in which the crossover value was investigated, the females, $\dfrac{w \quad M}{W^e \quad m}$, were all the daughters of one white-eyed female. The X-ray dose was practically the same in all cases but the duration of the treatment was varied from 3 minutes and 17 seconds to 20 hours and 20 minutes. The X-rayed and control females were transferred to new bottles every three days. The results are shown in an abbre-

viated form in the table from which one group of females which showed the same effect as the other two has been omitted. The first two sets of bottles show no significant difference in the crossover value between the control and the X-rayed females. The third and fourth bottles, however, show a significant difference between the control and X-rayed females both in the case of the separate groups and in the case of the series as a whole.

The counts of crossovers and noncrossovers from all of the experiments may be added together. It is then found that the difference between the crossover values of the control and X-rayed females for eggs laid during the first six days is 2.03 times the probable error and that for eggs laid during the second six to eight days of egg laying the crossover value of the X-rayed females is less than that of the controls by 28.37 times the probable error of the difference.

The fourth series of experiments shows that the effect of the X-ray treatment even when that treament lasts only for 3 minutes and 17 seconds is apparent in the crossover values over a period of approximately six days. This result tends to show that the X-rays do not directly affect the process of crossingover but that they produce in the chromosomes or the nucleus generally a condition which inhibits crossingover.

CROSSOVER VALUES—FOURTH SERIES OF EXPERIMENTS.

| Group | X-rayed | | | | Control | | | | |
	No. of Pairs	Noncross-overs	Cross-overs	% Crossing over	No. of Pairs	Noncross-overs	Cross-overs	% Crossing over	Diff. P. E. diff.
			FIRST	BOTTLES					
I	11	103	39	27.5	9	351	156	30.8	1.4
III	7	55	21	27.6	4	154	64	29.4	1.4
I-III	26	203	76	27.2	19	843	358	29.8	1.27
			SECOND	BOTTLES					
I	10	95	30	24.0	9	496	216	30.4	2.0
III	7	37	14	27.5	4	202	92	31.3	.8
I-III	24	173	59	25.4	18	1012	466	31.5	2.77
			THIRD	BOTTLES					
I	8	294	21	6.78	9	433	149	25.7	9.6
III	6	155	20	11.4	4	222	108	32.7	7.2
I-III	21	624	64	9.29	18	958	362	27.4	12.85

FOURTH BOTTLES

I	9	348	37	9.60	9	437	165	27.4	9.2
III	6	207	19	8.42	4	161	63	28.1	5.9
I-III	22	672	73	9.80	18	798	322	28.7	13.16

Group I was treated for 3 min., 17 sec. and Group III for 20 hrs., 20 min. Group II, not recorded separately in table, but included in the totals. Group I-III was treated for 2 hrs., 15 min. and gave results similar to those recorded for the other groups. All the groups received approximately the same dose, *i.e.*, total radient energy. The females, control and X-rayed, remained for three days in each bottle.

163 (2123)

Paramecium polycaryum, sp. nov.

By LORANDE LOSS WOODRUFF, and HOPE SPENCER.

[*From the Osborn Zoological Laboratory, Yale University, New Haven, Connecticut.*]

The several species of the genus Paramecium naturally fall into two groups: one with the general cell form represented by *P. aurelia, P. caudatum,* and *P. multimicronucleata* (aurelia group), and the other by *P. bursaria, P. putrinum* and *P. calkinsi* (bursaria group). Within each of these groups the species are distinguished chiefly by micronuclear structure and number. One type of micronuclear structure (caudatum type) occurs in *P. caudatum* and *P. bursaria,* and *P. putrinum;* the other (aurelia type) in *P. aurelia, P. multimicronucleata* and *P. calkinsi.* Species with the "caudatum type" possess a single micronucleus while those with the "aurelia type" possess two (*P. aurelia, P. calkinsi*) or several (*P. multimicronucleata*).[1]

The present paper records the discovery, on November 29, 1922, in some material collected in Louisiana, of a Paramecium characterized by the general body form of the "bursaria group"

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[1] L. L. Woodruff, *Biol. Bull.,* 1921, xli; PROC. SOC. EXP. BIOL. AND MED., 1921, xviii.

and the micronuclear structure of the "aurelia type," but possessing several (three to eight) micronuclei. In brief, the organism is essentially identical in form and structure with P. *calkinsi*, but has more than two micronuclei. Thus from the standpoint of micronuclear structure and number this animal holds the same position in the "bursaria group" as P. *multimicronucleata* in the "aurelia group."

Pedigree cultures from the original animal found have now been under observation and experimentation for nearly four months, and through upward of one hundred and fifty generations. During this time the animals have bred true, exhibiting the characteristic micronuclear number after emerging from the nuclear reorganization involved in endomixis.

In view of all the above data the animals of this culture are designated a new species, *Paramecium polycaryum*.[2]

164 (2124)

The effect of iletin (insulin) on the blood sugar content in adrenalectomized animals.

By G. N. STEWART and J. M. ROGOFF.

[*From the H. K. Cushing Laboratory of Experimental Medicine, Western Reserve University.*]

The existence of a close relationship between the adrenals, especially the medulla, and the pancreas in the regulation of the carbohydrate metabolism has been assumed by various writers. It seemed therefore of interest to determine whether the action of insulin on rabbits which had survived total adrenalectomy differed from its action on normal rabbits. No difference was made out. The adrenals were removed in two operations. The second adrenal was excised 3 weeks to 8 months before the insulin experiment. Six to eight blood specimens were obtained

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2 Details of the structure and life history of P. *polycaryum* will appear in the *Biological Bulletin*.

at intervals during 4 to 6 hours after subcutaneous injection of insulin. The blood sugar followed the course described by Macleod and his collaborators in normal rabbits, falling from initial values of 0.10 to 0.11 per cent. to a minimum of 0.039 to 0.048 per cent. (Folin-Wu method). When convulsions occurred, or the animal became comatose without going into convulsions subcutaneous injection of dextrose had the same markedly beneficial effect as in the normal control animals. Usually there was some rise in the rectal temperature (as much as 1.5° C. in a case where the animal became comatose without convulsions, and 2° C. in a case where convulsions occurred and dextrose was injected).

If the internal secretion of the pancreas and the internal secretion of adrenal medulla both take a share in the regulation of the blood sugar content (perhaps, as some have supposed, by exerting actions more or less antagnostic) it might appear not unlikely that each would influence the secretion of the other. We have accordingly made some experiments on the influence of insulin upon the output of epinephrin in the cat, in which animal the blood sugar curve after insulin runs much the same course as in the rabbit. Neither with subcutaneous nor with intravenous injection of insulin was any decided effect produced upon the output.

As evidence in favor of a relationship between the adrenals and the pancreas, it has been stated that after removal of the adrenals complete pancreatectomy does not produce hyperglycemia. This statement is based upon observations made on practically moribund animals. Dogs after complete pancreatectomy without interference with the adrenals show a hypoglycemia some time before death. Certainly the epinephrin from the adrenals has nothing to do with the development of hyperglycemia after pancreatectomy. The right adrenal was excised and the left denervated in a dog, and a month later total pancreatectomy was performed. The animal showed the typical picture of pancreatic diabetes, with blood sugar as high as 0.29 per cent. Two weeks after the pancreatectomy the left adrenal was removed. A few hours before this operation the blood sugar was 0.286 per cent.; 18½ hours after the operation it was 0.288 per cent.; 7 hours later 0.216 per cent. Two days after the operation it was only 0.08 per cent. and next day the animal was dead. In

this animal, 3 days after removal of the pancreas an experiment was made to determine whether insulin would affect the blood sugar content in the ordinary way in a dog whose epinephrin secretion was suppressed. The course of the blood sugar curve under the influence of insulin was the same as in a pancreatectomized animal not subjected to the adrenal operation (initial blood sugar 0.227; minimum reached in 4¾ hours 0.063 per cent.).

165 (2125)

The influence of iletin (insulin) on morphine hyperglycemia.

By G. N. STEWART and J. M. ROGOFF.

[*From the H. K. Cushing Laboratory of Experimental Medicine, Western Reserve University.*]

It has been shown by Macleod and his collaborators that insulin prevents the development of the hyperglycemia caused by etherization, asphyxia, carbon monoxide and piqûre, or counteracts it if already present. We have demonstrated that while these forms of experimental hyperglycemia are not essentially related to the adrenals, since they can be well elicited in the absence of those glands, this is not the case with the hyperglycemia produced by morphine in the development of which the adrenals seem to intervene in some way. We have therefore tested the influence of insulin upon morphine hyperglycemia in rabbits and cats. Whether morphine was given before, after, or at the same time as insulin, the characteristic effect of insulin upon the blood sugar was always observed. Thus, in a rabbit to which morhpine was administered 1 hour before insulin the blood sugar, which was 0.083 per cent. at the beginning of the experiment and 0.093 an hour after morphine, was 0.047 per cent. an hour after insulin had been injected. The morphine hyperglycemia had not had time to develop before insulin was given, nor did it develop in the 6 hours over which blood samples were collected. In another rabbit morphine was given an hour after insulin when the blood sugar had already fallen from 0.115 to 0.068 per cent.

It went on falling to 0.052 per cent, and never even regained the initial value.

In a normal cat morphine and insulin were administered at the same time. A marked hyporglycemia developed, the blood sugar sinking from 0.13 per cent. to 0.033 per cent. A similar experiment was performed on a cat whose right adrenal had been excised and the medulla of the left destroyed by a drill, the left gland being then denervated, 19 days before the experiment. The result on the blood sugar was the same as in the normal cat, the percentage falling from 0.103 to 0.040, with no attempt at return towards the initial value. The general symptoms were the same in the two cats, which presented a characteristic mixture of hyperexcitation (due to the morphine) and depression. In both animals the paradoxical pupil reaction was strongly marked in the left eye throughout the experiment (the left superior cervical ganglion had been previously excised), and quite as pronounced in the cat whose epinephrin secretion had been abolished as in the normal cat. The usual hyperthermia produced by morphine in cats was absent in both cases. Except for a slight temporary rise in the animal with the adrenal operation, the rectal temperature went on falling throughout the experiment. In this respect the insulin apparently caused the morphinized cats to behave like dogs or rabbits.

166 (2126)

The relation between chronic irritation of peritoneal mesothelium and the formation of adhesions.

By R. S. CUNNINGHAM.

[*From the Department of Anatomy, Johns Hopkins University, Baltimore, Maryland.*]

During a systematic investigation of the normal and pathological reactions of the peritoneal mesothelium, certain rather surprising facts have been revealed which are interesting because of their bearing on the question of adhesions.

Elsewhere[1] experiments have been reported in which rats re-

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[1] Cunningham, R. S., *Amer. Journ. of Physiol.*, 1922, lx, 448-460.

ceived repeated injections of solutions of glucose, the mesothelium undergoing certain changes of a morphological character which were not followed by adhesions. It seemed important to determine to what extent such changes could be produced without sufficient injury occuring to cause the development of adhesions. Such experiments have been carried out by the repeated introduction into the peritoneal cavity of various types of mild irritants, the best results having been obtained with laked heterogenous blood. Cats were given injections of 10 to 20 c.c. of laked rabbit's blood made isotonic with NaC1, the dose being repeated twice weekly over a period of 3 to 6 months. In such animals when the procedures were carefully guarded to prevent undue injury on puncture of the abdominal wall, and precautions taken to avoid any septic involvement, there resulted very remarkable changes in the entire mesothelial membrane without the formation of a single adhesion.

The peritoneum on section was often made up of two or three layers of cuboidal or columnar cells, attaining at times a thickness of 20 micra, and yet such a peritoneal lining seemed entirely adequate to prevent the formation of adhesions. From such observations the conclusion seems wholly justified that the presence of a complete layer of peritoneal lining cells, no matter how much their morphological appearance may be altered by such irritations as those used, is entirely sufficient to prevent the adherance of the two layers of peritoneum and thus prevent adhesions.

167 (2127)

Note on the permeability of the placenta in the rabbit.

By R. S. CUNNINGHAM.

[*From the Department of Anatomy, The Johns Hopkins University, Baltimore, Maryland.*]

The observations of various anatomists have shown very clearly that there are wide variations in the structure of the placental barrier in such species as the ungulate, the carnivora the rodentia, the chiroptera, and primates. Grosser[1] classifies

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1 Grosser, O., Vergleichende Anatomie und Entwicklungsgeschichte der Eihäute und der Pacenta, Leipzig, 1909, W. Braumüller.

the placentalia in terms of the layers of tissue which separate the maternal from the fetal blood-streams; but it must also be noted that the same layer of tissue may be of very different morphology in different species. In view of these differences it seems unfortunate that the vast majority of observations which have been made upon the passage of any substance from mother to fetus, or the reverse have been carried out on single species.

Among the few observers who have made comparative experiments on different species are Römer[2] using tetanus antitoxin, and Wislocki[3] using vital dyes. Römer found that the antitoxin passed readily in the human, occasionally in rodents, and never in sheep or cows. Wislocki found that trypan blue passed in small amounts in the rabbit and guinea-pig, but never in the cat and dog. In his discussion he suggests that both his and Römer's results may be dependant upon the varying complexity of the placental barrier in the animals studied.

Experiments have been reported elsewhere[4] in which it was shown that sodium ferrocyanide passed through the placental barrier in the cat from mother to fetus, while iron ammonium citrate was held up by some mechanism located in the fetal ectoderm. Further experiments have demonstrated that in the rabbit both the salts passed from mother to fetus, but it was found that the sodium ferrocyanide passed the placental barrier somewhat more easily than the iron ammonium citrate; this was shown by the fact that the ferrocyanide appeared in the fetal blood before the citrate, and always remained in greater concentration.

The placenta in the rabbit belongs to Grosser's hemo-chorionic type, while that of the cat is endothelio-chronic. But in addition to having an intact maternal endothelium the cat's placenta has a much thicker and more complex chorionic ectodermal layer than that found in the rabbit. It is possible that with the simplification of the placental barriers there is a decreasing amount of placental control; and a more widespread reduction of the activities of the placenta to the laws of osmosis and dif-

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[2] Römer, A., *Beitr. z. exper. Therap.*, 1904, H. 9.

[3] Wislocki, G. B., Contrib. to Embryol., No. 62, Carnegie Publ., No. 276, 89-101.

[4] Cunningham, R. S., *Amer. Jour. Phys.*, 1920, liii, 488-494.

fusion. Considering, however, all the evidence up to the present time, it seems probable that even in man the placenta does furnish some regulatory activity, while in some of the more complex types this control is much more elaborate.

These results with sodium ferrocyanide and iron ammonium citrate considered in connection with those of Römer and Wislocki indicate the advisability of extending, as far as possible, the investigation of each type of substance to as wide a group of different placental barriers as possible. This seems especially important in regard to those investigations involving the careful chemical estimation of the normal constituents of maternal and foetal bloods which so far have been studied chiefly in the human.

168 (2128)

Carbon dioxide and the HCO_3 ion as specific respiratory stimulants.

By ROBERT GESELL.

[*From the Washington University School of Medicine, St. Louis, Missouri.*]

It has been noted by Howell, Collip, Dale and Evans, the author and others that the intravenous injection of sodium bicarbonate may act as either a respiratory or circulatory stimulant, eliciting hypernea or a marked rise in the blood pressure. Such injection obviously increases the hydrogen ion concentration of the blood and inasmuch as it produces a slight dilution, it decreases the amount of carbon dioxide in the blood eliciting the stimulation. The increased respiration is, therefore contrary to the usually accepted laws of respiration. The only apparent change in the blood which might elicit stimulation is the greatly increased number of HCO_3 ions. Collip, therefore, suggests that the HCO_3 ion exerts a specific stimulating action on the respiratory center.

We believe, however, that this anomalous result may be otherwise explained. When the carbon dioxide is dissolved in water

it exists primarily in three forms—dissolved CO_2 molecules, dissolved undissociated H_2CO_3 molecules and dissociated H_2CO_3—thus $CO_2 \rightleftarrows H_2CO_3 \rightleftarrows H\ HCO_3$. Addition of sodium bicarbonate to the solution pushes the reaction to the left increasing both the dissolved CO_2 and the undissociated H_2CO_3. If we accept the view that the dissolved CO_2 and the undissociated H_2CO_3 diffuse freely into cells while the ions as such do not penetrate to any appreciable extent it is apparent that the injection of sodium bicarbonate increases the freely diffusable forms of carbon dioxide at the expense of the poorly diffusible ions and in that way increases the acid effects of the blood, at least on the interior of the cells (and possibly in the lymph bathing the cells as will be discussed in a later paper) even though the actual sum total of the original dissolved carbon dioxide in its various forms is not increased and the hydrogen ion concentrate of the blood is actually decreased. This alone should result in the diffusion of carbon dioxide against a positive gradient. That is, the injection of sodium bicarbonate should theoretically tend to produce acidosis along with alkalemia by the accumulation of carbon dioxide in the tissues. The hyperpnea, the rise in blood pressure and the shortening of apnea artificially produced by forced ventilation suggests that a slight degree of acidosis is actually produced.

But this view neglects entirely the effects of the H and HCO_3 ions. The justification of this perhaps might be questioned. In so far as lactic acid appears to diffuse in the dissociated condition from the tissues to the blood there appears to be no reason why the dissociated carbonic acid should not also diffuse.

Arrhenius showed that the addition of a salt to a solution of its acid increased the diffusion of the acid into water solution, for example the addition of sodium chloride to a solution of hydrochloric acid increases the rate of diffusion of hydrochloric acid. The phenomenon depends on the increase of the common anion, Cl and the relative mobility of the cation H and Na.

By means of a simple diffusion experiment without the aid of a membrane and with the use of indicators the diffusion of acid from a point of lower concentration to a point of higher concentration can be demonstrated to occur within a few seconds. This prenomenon would appear to be of considerable significance.

It is obvious that the conditions are somewhat different in the case of a weak acid such as carbonic acid and its salt, sodium bicarbonate. Carbon dioxide occurs in solution in three forms and the relative amounts are varied by the addition of sodium bicarbonate. Though the acid effects of the solution on the interiors of cells placed in such solution are theoretically increased by the increase in the freely diffusible CO_2 and H_2CO_3 molecules, the number of free hydrogen ions is diminished. Yet the effectiveness of these ions is greatly increased by the increase in the common anion HCO_3. If a differential barrier, impeding the migration of the sodium ion, exists between the blood and site of action of the hydrogen ion in the respiratory center a double mechanism exists for the passage of carbon dioxide from the point of low to a point of high concentration accompanied by increased acidity. Michaelis calls attention to the fact that H and OH ions are adsorbed at phase boundaries more than other ions and also that the addition of salts to solution of acids or bases increases this adsorption. This phenomenon should also be carefully considered in relation to the hydrogen ion concentration at the site of stimulation. We hope to show in a later paper the relative importance of the various salt effects of sodium bicarbonate.

In connection with the specificity of carbon dioxide, we have by means of simple diffusion experiments without the aid of a membrane demonstrated striking effects on the hydrogen ion concentration of carbonate buffer solutions on each other, dependent solely on the differences in concentration of carbon dioxide and sodium bicarbonate and their relative rates of diffusion. A relatively alkaline solution of high carbon dioxide tension exerts an acid effect even on a relatively acid solution of lower carbon dioxide tension. The results are comparable to those obtained by Jacobs in the living cell and in the model cell with a lipoid solvent membrane. Our results, however, since they are obtained without a membrane are independent of any peculiar property of the cell membrane. They suggest the possible significance of the relative rates of diffusion, of the numerator and the denominator of the buffer mixture not only in buffer solutions outside the cell, but possibly inside as well.

As Fletcher and Hopkins have shown that the rate of escape of carbon dioxide from saturated living muscle, dead muscle,

egg albumin and water follows a similar curve, our experiments further suggest that the acid effects of alkaline buffer solutions with high carbon dioxide tension on living cells depend rather on the relative impermeability of the membrane to the metallic cations preventing free entrance of the sodium bicarbonate than on a specific solubility of carbon dioxide in the cell membrane.

Abstracts of Communications.

Tenth meeting.

Minnesota Branch, Minneapolis, Minnesota, March 14, 1923.

169 (2129)

The mechanism of serum fastness.

By W. P. LARSON and RUTH GREENFIELD.

[*From the Department of Bacteriology, University of Minnesota, Minneapolis, Minnesota.*]

Soon after the discovery of the agglutinins it was observed that some strains of a given microorganism were not agglutinated by a specific immune serum. Such non-agglutinable strains are said to be serum fast. The mechanism of serum fastness is not well understood. Ehrlich's explanation of this phenomenon on the basis of suppressed receptors does enable us to visualize the condition, but a suppression of receptors is probably far from what actually takes place.

In our studies[1] on pellicle formation it has been shown that pellicle forming bacteria are rich in acetone-ether soluble substances. Bacteria which ordinarily do not grow in pellicle were caused to do so by growing them in broth to which had been added glycerine or carbohydrates which they did not ferment. By growing the staphylococcus on glycerine broth for a few generations it began to grow in a pellicle, and finally formed as

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[1] *Jour. Inf. Dis.*, 1922, xxxi, 407-415.

definite a pellicle as the tubercle bacillus, or other pellicle form-
ing organisms.

The acetone-ether soluble substances of staphylococci grown
in this way increased from 7.9 per cent. to 39.9 per cent.

It was concluded that pellicle formation was due to the in-
creased amounts of acetone-ether soluble substances by virtue
of which the bacteria resist wetting, and are thus supported on
the surface of the medium by surface tension.

The present study concerns the effect of wetting on the agglu-
tination reaction.

A laboratory strain of the staphylococcus aureus was grown
in parallel cultures on ordinary broth and three per cent.
glycerin broth respectively. Rabbits were immunized with
killed cultures of the staphylococcus grown on ordinary broth.
In the interest of brevity the strain grown in ordinary broth
will be referred to as the "lean" strain and that grown in glycerine
broth as the "fat" strain. An agglutinating serum was thus ob-
tained which agglutinated the lean strain in a dilution of 1-90.

Parallel agglutination tests were then made with the fat and
lean strains in serum dilution of 1-50. After four hours in the
incubator the lean strain was completely precipitated, while there
was no visible change in the tests with the fat strain. However,
after 30 hours there appeared to be some precipitation. At this
point a count was made to determine the percentage of bacteria
still remaining in suspension as compared with the control,—
which contained the same number of bacteria without the serum.
The count was made by a laboratory worker not personally in-
terested in the experiment. The count revealed that 30 per cent.
of the organisms had been precipitated by the serum, while 70
per cent. still remained in suspension. The test tube containing
the fat strain which had been in contact with the agglutinating
serum for 30 hours was then centrifuged and the supernatant
fluid removed, and to this supernatant fluid was added the proper
amount of the lean strain. Agglutination was found to be
prompt. This experiment indicates that there had been very
little adsorption of the antibodies by the fat strain.

The fat strain was then cultivated on ordinary broth, making
daily transplants, and the agglutinability of each generation
tested. It was found that the agglutinabiliy of the fat strain
was completely restored after three generations of culture on or-

dinary broth. The first generation on ordinary broth showed a tendency to agglutinate, but the reaction was much slower than in the control test. The experimental production of serum fast staphylococci suggests that the mechanism of serum fastness may be due to a lack of wetting. This would then explain why the tubercle bacillus which is so rich in fat-like substances gives inconstant serum reactions.

170 (2130)

The precipitin test in the diagnosis of tuberculosis.

By W. P. LARSON, IRWIN A. MONTANK, and EDMOND NELSON.

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The precipitin test has been found to give reliable results in the diagnosis of active tuberculosis. The antigen is prepared by disrupting tubercle bacilli, preferable an old culture, with carbon dioxide by the method described by Larson, Hartzell and Diehl.[1] The disrupted bacteria are filtered through paper in order to remove the shells. The clear filtrate is layered over the serum to be tested, and the tubes incubated for a period up to two hours. A definite cloudy ring at the interface of the two fluids indicates a positive reaction. The cloud often appears within the first five minutes. In the far advanced cases, however, the reaction develops more slowly, but is usually very definite. Upon standing several hours the ring gradually becomes dispersed.

Thus far the blood serum of 190 cases have been examined. Of these, 100 were patients in the University Hospital and Dispensary, but not in the tubercular clinics. Ninety cases, representing all stages of tuberculosis, were from a local sanatorium. From the 100 cases not suspected of having tuberculosis, eleven positive reactions were obtained. Six of these have since been

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[1] *Jour. Inf. Dis.*, 1918, xxii, 271.

found to have evidence of tuberculosis. One died from hypertension, and at autopsy an active lesion was found in the apex of the right lung.

An interesting case was that of a newborn, whose mother was a far advanced case, giving a negative Von Pirquet but a positive precipitin test. The blood of the newborn gave a heavy precipitin reaction.

Of the 90 sanatorium cases 85 were positive and 5 negative. The 5 negative cases were either "healed" or "arrested."

We have found that the acid fast actinomycetes, as A. *gypsoides* and A. *asteroides,* make a good antigens as the tubercle bacillus. On the other hand the non-acid fast actinomycetes fail to react with tubercular serums. This is in agreement with the findings of Henrici and Gardner[2] and Nelson and Renrici.[3]

The glycerine broth filtrate of the tubercle bacilli as of the actinomycetes fails to give this reaction.

171 (2131)

The determination of iodin in iodin metabolism.

By J. F. McCLENDON.

[*From the Laboratory of Physiologic Chemistry, University of Minnesota, Minneapolis, Minnesota.*]

The organic material for iodin analysis is dried, mixed with and covered with CaO to render it alkaline and reduce the rate of combustion, and burned in pure oxygen in a large combustion tube with the end narrowed and bent downward for about 50 cm. The first third of this narrowed portion is covered with a thin layer of asbestos fibers to protect it from a lead coil through which cold water runs. The middle third is water-jacketed. The lower third dips into an absorbing apparatus filled with NaOH solution. The greater the amount of CaO mixed with the un-

2 *Jour. Inf. Dis.*, 1921, xxviii, 237-248.

3 PROC. SOC. EXP. BIOL. AND MED., 1921, xix, 351-352.

known material, the greater amount of iodin remains in the ash. The alkaline solution from the absorption apparatus is evaporated to 10 c.c. after adding to it the rinsings of the combustion tube. The ash is ground with water or the solution from the absorption apparatus in a ball mill and the extract is evaporated to 10 c.c. The solution is acidified until $P_H = 1$ and placed in a 12 c.c. separatory funnel and 0.1 c.c. of 0.1 N arsenius acid added, and allowed to remain for one-half hour to reduce any iodate to iodide. Then 0.1 c.c. of 5 per cent. sodium nitrite is added to oxidize the iodide to iodin which is extracted with 1 c.c. of CCl_4. The partition coefficient of iodin between CCl_4 and the aqueous solution is about 86, but varies with conditions. The iodin in CCl_4 is run into a 1 c.c. glass stoppered bottle and centrifuged to remove water droplets. The iodin is determined colorimetrically with a Bausch and Lomb Biological colorimeter specially made with cups holding 1 c.c. at 2 cm. depth, against a standard solution of pure iodin in CCl_4 (1 mg. in 10 c.c). The amount remaining in the aqueous solution may be calculated, using an approximate partition coefficient, or it may be recovered by repeated extractions. CCl_4 is freed from reducing substances by oxidation with bromine in the sunlight for about a week; then the excess Br is removed by shaking with a dilute solution of KI and then titrating the water phase with sulphurous acid while shaking the container. It is then washed and filtered (and preferably dried) and distilled, rejecting the first and last portions of the distillate. Standard solutions of iodin in CCl_4 cannot be sealed by fusing the glass without decolorizing. Preserved in glass stoppered bottles it will turn yellow. The color may be regenerated by shaking with 1/10 its volume of water of $P_H = 1$ containing nitrous acid, but some of the iodin is removed. This water may then be used to regenerate new portions of standard solution without appreciable loss.

By determining the iodin in the ash separately from that caught by the absorption apparatus, the amount of CaO that must be added to make the absorption apparatus unnecessary may be determined, but under such conditions the combustion is slow.

By this method it was determined that bacteria remove iodin from the medium.

172 (2132)

Simple empirical formulae for expressing the lineal growth of the human fetus.

By RICHARD E. SCAMMON and L. A. CALKINS.

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In the quantitative study of the growth of the human body there is often need for simple and accurate formulæ for expressing the relation between body-length and prenatal age. Empirical formulæ for lineal growth in the fetal period (3 lunar months to birth) have been published by Hassee[1], Henry and Bastien[2], and Scammorn[3]. No one of these is entirely satisfactory, for the first gives results which are not in accord with modern findings regarding prenatal lineal growth, the second is highly complicated, and the third is primarily for use in estimating the length from the age, whereas one usually desires to determine the age from the length. Practicable formulæ of this kind should fulfill the following conditions: (a) They should express age in terms of body-length for the entire fetal period (3 months to birth). (b) They should give a close fit to reliable data on the subject. (c) They should be in simple form, which will permit their application with ordinary arithmetic without the use of tables of special functions and the like. With these conditions in mind the following formulæ have been developed on the basis of the data of Mall[4].

When Mall's observations are placed in graphic form they approximate a parabola having the general form: $T = a + bL + cL^2$, where T is the age in fetal or lunar months (calculated from the first day of the last menstruation), L is the total or crown-heel length in cm., and a, b and c are constants. The specific formula for expressing this relations is:

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1 Hasse, *Charité Ann.*, 1875, ii, 669.

2 Henry et Bastien, *C. R. Acad. Sc.*, 1904, cxxxix, 811.

3 Scammon, PROC. SOC. EXP. BIOL. AND MED., 1921, xix, 133.

4 Mall, Determination of the age of human embryos and fetuses; Keibel and Mall, *Human Embryology*, 1910, i, 199.

$$(1) \quad T = 2.3 + \frac{2.5\ L}{28} + \frac{L^2}{784}.$$

This may be simplified to :

$$(2) \quad T = (\frac{L}{28} + 1.25)^2 + 0.74.$$

For estimating the crown-heel length from the age, the formula may be transformed into :

$$L = 28\sqrt{T - 0.74} - 35.$$

These formulæ may also be modified for further use. According to Mall, the cohabitation age averages 10 days less than the menstrual age which is estimated from the first day of the last menstruation, and the individual deviations from this average are quite small. Therefore, the formula for menstrual age may be modified to

$$(3) \quad T = 1.94 + \frac{2.5\ L}{28} + \frac{L^2}{784}$$

or

$$(4) \quad (\frac{L}{28} + 1.25)^2 + 0.38$$

to express the cohabitation age.

While the above formulæ are not complicated, somewhat simpler ones may be used for approximate values. These are

$$(5) \quad T = (-\frac{L}{30} + 1.49)^2$$

for estimating age from total length and

$$(6) \quad L = 30\sqrt{T} - 44.7$$

for estimating length from age.

The values given by these various formulæ and their absolute and percentage deviations from Mall's observed values are shown in the accompanying tables.

TABLE I.

Observed and Calculated Body-length of the Human Fetus by Fetal Months.

Age in fetal months	Total body-length by interpolation from Mall's data (a)	Total body-length calculated by exact formula. (b)	Total body-length calculated by approximate formula (c)	Deviation of "b" from "a"		Deviation of "c" from "a"	
				cm.	Per cent.	cm.	Per cent.
3	7.06	7.08	7.26	+0.02	+0.28	+0.20	+2.97
4	15.55	15.54	15.30	−0.01	−0.07	−0.25	−1.67
5	22.53	22.79	22.38	+0.26	+1.15	−0.15	−0.66
6	29.40	29.20	28.79	−0.20	−0.68	−0.61	−2.01
7	35.00	35.05	34.67	+0.05	+0.14	−0.33	−0.94
8	40.83	40.43	40.15	−0.40	−0.98	−0.68	−1.42
9	45.40	45.47	45.20	+0.07	+0.15	−0.20	−0.49
10	50.00	50.20	50.17	+0.20	+0.40	+0.17	+0.34
			Sum	1.21	3.85	2.59	10.50
			Mean	0.15	0.48	0.32	1.31

TABLE II.

Observed and Calculated Age of the Fetus at 5 cm. Intervals of Body-length.

Total (crown-heel) body-length (cm.)	Age in fetal months by interpolation from Mall's data (a)	Age in fetal months as calculated by exact formula (b)	Age in fetal months as calculated by approximate formula (c)	Deviation of "b" from "a" Fetal months	Deviation of "b" from "a" Per cent.	Deviation of "c" from "a" Fetal months	Deviation of "c" from "a" Per cent.
5	2.71	2.78	2.72	+0.07	+2.58	+0.01	+0.36
10	3.38	3.32	3.29	−0.06	−1.77	−0.09	−2.66
15	3.92	3.93	3.96	+0.01	+0.25	+0.04	+1.02
20	4.605	4.61	4.62	+0.005	+0.10	+0.015	+0.32
25	5.36	5.35	5.38	−0.01	−0.18	+0.02	+0.37
30	6.08	6.03	6.20	−0.05	−0.82	+0.12	+1.97
35	6.95	6.99	7.02	+0.04	+0.57	+0.07	+1.01
40	7.82	7.90	7.95	+0.08	+1.02	+0.13	+1.66
45	8.905	8.90	8.99	−0.005	−0.05	+0.85	+0.95
50	10.00	9.95	9.93	−0.05	−0.50	−0.07	−0.70
			Sum	0.38	7.84	0.65	11.02
			Mean	0.038	0.78	0.065	1.10

SCIENTIFIC PROCEEDINGS

173 (2133)

Chemical changes in the blood in intestinal obstruction.

By J. F. CONNORS, J. A. KILLIAN and H. B. EISBERG.

[*From the Surgical Service of Harlem Hospital, Departments of Biochemistry, New York Post-Graduate Medical School and Hospital, and of Experimental Surgery, University and Bellevue Hospital Medical College.*]

For the past two years an intensive study has been made of the non-protein nitrogen of the blood and some of its components, in various pathological conditions. The results obtained in pneumonia and the toxemias of pregnancy have been described by one of us (K) [1, 2]. This communication is an extension of that study, presenting observations on similar blood changes in patients with intestinal obstruction and a duplication of the findings in experimental animals. The studies were completed in 1921. A number of articles have been contributed to the literature reporting somewhat analogous findings. In 1916 Whipple, Rodenbaugh and and Gilgore[3] isolated from closed intestinal

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[1] Killian, J. A., *Amer. Jour. Obst. and Gyn.*, 1921, ii, 6.

[2] Killian, J. A., *Proc. N. Y. Path. Soc.*, 1922, xxii, 72.

[3] Whipple, G. H., Rodenbaugh, F. H., and Kilgore, A. R., *Exper. Med.*, 1916, xxiii, 123.

loops toxic compounds closely resembling primary proteoses in all their properties. Cooke, Rodenbaugh and Whipple[4] have shown that in cases of acute intestinal obstruction, whether produced experimentally in animals or occurring in human cases, is accompanied by a rapid rise in the non-protein nitrogen of the blood, from 3 to 10 times normal figure. However, in chronic cases there may be little or no increase in the blood non-protein nitrogen. These intoxications also show an increased blood content of urea and creatinine. The authors state that the kidneys in all of these cases are practically normal (anatomically) and that the protein or tissue destruction, rather than impaired renal function, is responsible for the rise in the blood nitrogen. Rabinowitch[5] reports a significant increase in the urea nitrogen of the blood in patients with intestinal obstruction. In many cases it rose to more than 100 mg. per 100 c.c With this rise in urea nitrogen the author noted a normal phenolsulphonephthalein excretion by the kidneys, and he ascribed the increased urea nitrogen to tissue destruction. Haden and Orr[6] noted after ligation of the duodenum, ligation of the duodenum with gastroenterostomy and a ligation of the upper half of the ileum in dogs, a rise in the non-protein and urea nitrogen and CO_2 combining power of the blood, but a fall in the chlorides. The uric acid, creatinine, amino acid nitrogen and sugar remained unchanged. Ligation of the ileum at the ileocecal valve is followed by but a slight increase in the nitrogen, and no change in the CO_2 combining power. These authors believe that the blood urea nitrogen is a good index of the protein destruction.

At present report we are including only data on non-protein nitrogenous compounds of the blood, the sugar and CO_2 c.p. Hiller and Van Slyke[7] in a recent paper have shown after a comprehensive study of effect of the various protein precipitants used in blood analysis upon the constituents of the non-protein nitrogen, that precipitation of the proteins with 2.5 per cent. trichloracetic acid is the preferable method in analyses concerned with the undetermined nitrogen. Our procedure has been to

4 Cooke, J. V., Rodenbaugh, F. H., and Whipple, G. H., *Exper. Med.*, 1916, xxiii, 717.

5 Rabinowitch, I. M., *Canad. Med. Assoc. J.*, 1921, xi, 163.

6 Haden, R. L., and Orr, T. G., *J. Exper. Med.*, 1923, xxxvii, 365 and 377.

7 Hiller, A., and Van Slyke, D. D.; *Jour. Biol. Chem*, 1922, lii, 253.

dilute the blood 1-5 with water and after laking has been completed, to precipitate the proteins with an equal volume of 5 per cent. trichloracetic acid. The acid then has a concentration of 2.5 per cent. of the total volume.

In simple but complete experimental intestinal obstruction the first change noted in the blood was a rise in the non-protein nitrogen from 36-90 mg. This rise is more rapid, the nearer the obstruction is placed to the duodenum, where the toxemia is most severe, indicating a corresponding increase in the rest-nitrogen, Since the dogs are on a water diet, this nitrogen must be of endogenous origin. A similar rise in non-protein nitrogen is noted in the segmental type of obstruction. However, this rise is much greater and more rapid than simple obstruction occurring at the same level. In but one animal the urea nitrogen rose above normal limits, 21.5 milligrams. The non-protein nitrogen, however, was 47 milligrams which was more than twice the urea nitrogen.

All the animals showed a decrease in the alkaline reserve as indicated by the carbon dioxide combining power. In obstructions of the segmental type the drop in the carbon dioxide combining power is more marked.

In the clinical cases reported, an increase in the non-protein nitrogen from 36 to 83 milligrams occurred. This is in accord with the experimental findings. In many cases the urea nitrogen is above normal, but it forms less than fifty per. cent. of the non-protein nitrogen. The uric acid is definitely increased above normal 4-11 mg. per 100 c.c. Following operation, with relief of obstruction in most instances there is a steady decrease in non-protein nitrogen. This decrease in non-protein nitrogen was found to be associated with a clinical improvement in the patient's condition.

In one case there was a rapid rise in non-protein nitrogen following operation from 51 to 83 mg. in 4 days. Following a stormy convalescence the patient recovered. In this instance several feet of gut with questionable viability were returned to the peritoneal cavity. The increase in urea, uric acid and creatinine of the blood appears to be subsequent to a rise in the non-protein nitrogen.

When there is an accumulation of the nitrogenous waste products they follow the order of retention characteristic of renal impairment, namely a successive rise in uric acid, urea nitrogen and creatinine.

CONCLUSIONS

The results obtained above indicate that the extent of the toxemia is related to the level of the non-protein rather than the urea nitrogen. The toxemia is dependent upon the location of the obstruction in relation to the duodenum and the type of the obstruction, whether simple or segmental. From the practical standpoint the chemical examination of the blood is of inestimable value in pre-operative diagnosis and prognosis.

174 (2134)

Cystine metabolism.

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In our study of cystine and cystein we have had in mind four different problems.

1. An attempt to synthesize cystine in the animal body either from endogenous nitrogen and sulfur or from these same elements when fed in different forms. This could not be accomplished as has already been shown.[1]

2. We prepared several compounds of cystine and cystein[2] where first, the amino group was blocked by some radical such as the phenylacetyl or phenyluramino,—then both the amino group and the carboxyl as in the phenylhydantoin derivative,—then a blocking of the S group with a benzyl radical followed by a blocking of both S-H and amino group, and lastly a blocking of these two and the carboxyl group.

3. We have fed these compounds in order to determine whether the blocking of one or more of these groups prevent the oxidation of the cystine or cystein molecule. Besides this we

1 Muldoon, J. A., Shiple, G. J., and Sherwin, C. P., PROC. SOC. EXP. BIOL. AND MED., 1922, xx, 46.
2 Shiple, G. J., and Sherwin, C. P. (in press), *J. Biol. Chem.*, 1923, lv, 671.

wished to investigate the reactions by which cystine is apparently converted into cystein, and cystein into cystine in the body.

4. In studying the catabolism of cystine we decided to determine, if possible, the origin of the ethereal sulfates.

Lewis[3] has found that the sulfur of phenyluramino cystine is not to any great extent oxidized. He has shown that the compound is however apparently split into phenyluramino cystein, as he obtains a decided test with sodium nitroprusside, and ferric chloride, on the urine of rabbits after feeding the phenyluramino cystine.

Schmidt[4] has shown that taurine, $CH \cdot NH_2 \cdot CH_2 \cdot SO_3H$, is not catabolized in the body but passes out in the urine unchanged. Cysteic acid, $CO_2 \cdot HCH \cdot NH \cdot CH_2 \cdot SO_3H$, only undergoes hydrolytic deamination but not oxidation in the body.

We have fed to rabbits phenylacetyl cystine, phenyluramino cystine, phenylhydantoin cystine as well as cystine itself.

In these derivatives of cystine either the amino group alone or the amino and carboxyl group were both blocked, and our results have shown that a protecting of the amino group does not seem to entirely protect the sulfur, as considerable of it is excreted as sulfates.

We fed on each of three succeeding days phenylacetyl cystine to one rabbit and phenyluramino cystine to another rabbit in such amounts that each rabbit received 0.4 grams sulfur. Only a small amount of the phenylacetyl cystine could be traced, as most of it was completely lost. Enough of the original substance was recovered and isolated to determine the melting point and also some of it was reduced to the corresponding cystein compound as shown by the sodium nitroprusside test, but attempts to convert this phenylacetyl cystine into benzylphenylacetyl cystein, for its further identificaion were impossible.

In the case of the phenyluramino cystein, we were able not only to isolate some of the substance fed, but proved the presence of the corresponding cystein compound by extracting it from the urine and converting it into the benzyl derivative which

3 Lewis, H. B., and Root, L. E., *J. Biol. Chem.*, 1922, 1, 303; Lewis, H. B., and McGinty, D. A., *Ibid*, 1922, liii, 349.

4 Schmidt, C. L. A., Adelung, Von Aldelung, E., and Watson, T., *J. Biol. Chem.*, 1918, xxiii, 501; Schmidt, and Allen, E. G., *Ibid*, 1920, xlii, 55; Schmidt, and Clark, G. W., *Ibid*, 1922, liii, 193.

we had previous prepared. We next fed phenyluramino cystine which we had synthesized, to rabbits, to see whether this compound would be excreted or some of it changed into the cystine derivative. We found that much of it had been oxidized to the cystine compound.

This shows that the reaction

$$Cystin \rightleftharpoons Cystein$$
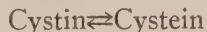
may be a common metabolic reaction and easily as well as precisely controlled by the living cell. It is interesting in as much as it is closely allied with the finding of Hopkins[5] on his glutathione work and is strongly corroborative.

175 (2135)

A micro colorimetric method of estimating the hydrogen ion concentration of the blood.

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A discussion of the bicolorimetric principle was first presented to this Society in November, 1921,[1] at which time we had the present work in mind. The method described below is essentially an adaptation of the colorimetric method of Cullen[2] for the determination of the P_H of the blood plasma (or serum) to the bicolorimeter described by one of us.[3] As modified the final determination is carried out on 0.1 c.c. of plasma, and does not require more than 10 minutes after the blood has been obtained. The color comparison can be made with an accuracy of $\pm P_H$ 0.02.

5 Hopkins, F. G., *Biochem. Jour.*, 1921, xv, 286.

* Medical Fellow of the National Research Council.

1 Myers, V. C., PROC. SOC. EXP. BIOL. AND MED., 1921, xix, 78.

2 Cullen, G. E., *J. Biol. Chem.*, 1922, lii, 501.

3 Myers, V. C., *J. Biol. Chem.*, 1922, liv, 675.

Blood is drawn without stasis in a narrow 5 c.c. Luer glass syringe containing sufficient mineral oil to fill any air spaces, and is at once delivered into a centrifuge tube of special design under oil. This tube is made of Pyrex glass and has at the bottom a bulb of 2 c.c. capacity (30 mm. in length with an internal diameter of 11 mm. and a neck of 4 mm.). Tubes with bulbs of 1 and 5 c.c. capacity have also been used, the latter being employed when a simultaneous estimation of the CO_2 content of the plasma is to be made. One drop of neutral 20 per cent. potassium oxalate is dried in the tube, after which three drops of mineral oil are added. In transferring the blood from the syringe to the centrifuge tube, the point of the needle is placed under the oil and sufficient blood delivered to bring the oil into the neck of the bulb. With the slight pressure exerted the blood readily takes up the oxalate and does not clot. The tube is centrifuged at moderate speed for about two minutes to separate the plasma.

As a check on the possible influence of the oil on the P_H under these conditions, specimens of blood plasma, saturated with CO_2 at alveolar tension, have been similarly centrifuged under mineral oil. P_H estimations were made both before and after the centrifuging, but disclosed no appreciable change in the P_H.

A 0.9 per cent. solution of sodium chloride in CO_2 free water, to which has been added 10 c.c. of 0.02 per cent. phenol red solution for each 100 c.c., is adjusted to a P_H of 7.4 with sodium hydroxide and then kept under oil in a paraffin lined bottle. Two c.c of this solution are allowed to flow into the cup of the bicolorimeter under oil. A small portion of the separated plasma is now drawn into a 0.5 c.c. tuberculin syringe graduated in 0.01 c.c. (the point of the needle can best be cut off), the air spaces of which are filled with mineral oil. One tenth c.c. of the plasma is immediately discharged into the saline solution in the cup. This solution is stirred with a small glass rod and is then ready for color comparison.

For this purpose the two wedges of the colorimeter are filled with Sörensen's buffer phosphate solutions, containing 2 c.c. of 0.02 per cent. phenol red for 20 c.c. of phosphate solution, the front wedge having a P_H value of 8.0 and the second wedge of 6.8. The wedges are calibrated, as already described,[2] with a series of buffer phosphates ranging from P_H 7.0 to 7.8 and differing by 0.1 P_H. The readings made with the wedge con-

taining the dominant color (alkaline, P_H 8.0) are employed in plotting the curve from which the calculations are made. Although the solutions apparently keep for some time in the wedges, they should be checked at frequent intervals.

In several instances where the plasma has been slightly cloudy a third wedge containing finely suspended barium sulfate has been employed to equalize the fields. In this way perfect color matches have been obtained.

The factors worked out by Cullen to correct the P_H values to body temperature (38°C) have been employed.

FIGURES FOR THE P_H AND CO_2 CONTENT OR COMBINING POWER OF THE BLOOD PLASMA IN SEVERAL NORMAL AND PATHOLOGICAL CASES

Case	Age	Sex	Date 1923	P_H	CO_2 combining power	Diagnosis, remarks
					c.c. per 100	
1. A. S.	60	M	4/13	7.37	65	
2. J. S.	9	M	4/13	7.40	63	Normal findings.
3. M. M.	30	M	4/13	7.41	67	
4. S. G.	22	M	3/28	7.23	22	Diabetic coma.
			3/28	7.33	28	4 hrs. after insulin.
			3/29	7.275	30	Next morning.
			4/18	7.35	47*	
5. J. B.	6	M	3/30	7.21	21	Diabetic coma.
6. R. M.	45	M	4/5	7.15	20	Uremia following bladder operation.
7. W. T.	35	M	4/16		31	Chronic nephritis.
			4/17	7.30	40*	
8. E. L.	47	F.	4/20	7.30	63*	Cardiac decomposition.
			4/23	7.40	63*	
9. N. M.	52	F	4/9		98	Cholecystectomy, had received 40 gm. $HNaCO_3$, second specimen on 4/9 taken 6 hrs. after first.
				7.53	87	
					83*	
			4/10	7.46	79	
					79*	

* CO_2 content.

In a series of 25 miscellaneous hospital cases, in which abnormal value for the P_H were not anticipated, the plasma figures obtained with the method described varied between P_H 7.35 and 7.43, with an average close to 7.39. Illustrative figures on three cases with normal findings and on several interesting pathological conditions are given in the table. In two cases of diabetes in coma the P_H values were 7.23 and 7.21, the figures

harmonizing with the CO_2 combining power in both instances. In the first of these cases there was a rise to P_H 7.33 four hours after administration of insulin. In case 6 suffering from uremia following a bladder operation, and dying several hours after the test, the P_H was 7.15 and the CO_2 20. In case 7 suffering from advanced nephritis with marked nitrogen retention (blood creatinine of 9 mg.), the P_H was 7.30 and the CO_2 content 47. In the case with cardiac decompensation there appears to be a slight reduction in the P_H. Case 9, following a cholecystectomy operation and alkali therapy, showed a CO_2 combining power of 98. Six hours later when a P_H of 7.53 was observed the CO_2 had fallen to 87. On the next morning the P_H was 7.46 and the CO_2 was 79.

176 (2136)

Some studies on the vital staining of blood cells.

By R. SPIRIDONOVITCH (by invitation).

[*From the Department of Anatomy, Cornell University Medical College, New York City.*]

There are many papers on the vital staining of the blood cells but, considering the many papers of the conventional method used in the examination of the blood, one may say that the field of the vital staining is rather unexplored. It is surprising to find how few have actually studied living cells. Most of the work on vital staining is in reality supra vital, that is to say the living cells on taking up the stain died. I will mention of many investigators the names of Rosin and Biebergeil, Sabin and E. Cowdry. These writers observed cytoplasmic granules which took up certain dyes, while the cells containing them continued to live.

I have made a few comparative studies of the effect of certain vital dyes on the cytoplasmic granules in the white blood cell of man. To introduce the dye into a drop of blood diluted with Ringer's solution, I used the following method, which was

suggested to me by Mr. Chambers: Coverslips were flooded with an aqueous solution of the dye, which was allowed to evaporate, leaving the dry dye evenly distributed on the cover-slip. The drop of blood was placed on this coverslip and studied as a hanging drop, suspended in culture slides. The observations were made in a warm chamber.

Out of a dozen various dyes I have found the two following to be the best. They are both American made dyes. Janus Green from the Providence Chemical Laboratories and Cresylecht Violet from the National Aniline and Chemical Co. This American Janus Green is taken up very rapidly. During the first few seconds it colors the cytoplasm diffusely and gradually concentrates on the granules, which finally take up all of the stain, leaving the cytoplasm almost transparent. The eosinophil granules are very prominent and large and take a deep blue color. During this time the cell exhibits amoeboid movements. The granules move but seldom entend into the slender pseudopodia.

After one hour, as the cell begins to die, the Janus Green tends to fade out of the granules and enters the hitherto colorless nucleus, coloring it violet. This is significant because it indicates a reduction of the dye. The neutrophil leucocytes have very fine granules which stain more faintly. These granules exhibit greater activity of movement than those of the cosinophiles. In dead cells, both eosinophil and neutrophil, the nucleus loses its color while the granules again take up the stain and persist as deeply stained granules for a long time.

Cresylecht Violet is more toxic than Janus Green. Its preliminary effect, however, is to stimulate not only the cells but also the cytoplasmic granules to considerable activitiy.

In the neutrophil cells I have found two distinct kinds of granules, first, one which is apparently identical with that of the eosinophil and second, one which is very small. The large granules may be few or many. Although these granules resemble the eosinophil granules, we cannot consider them as being the same, because, during the decoloration of the cell which accompanies its death these large granules fade out at the same time and in the same way, as the fine neutrophil granules and, therefore, earlier than the granules of the eosinophil cell.

The staining of the living cells depends on many physical conditions. Such a small variation between 0.6 per cent. and 0.65 per cent. of NaCl in Ringer's solution makes a great difference in the vital staining process. The temperature is also important for it seems that each color, to give the best results, must be used at a particular temperature, for instance Cresyl Violet gives best results at the low temperature of 20°C., Diazin Green and Janus Green at 26°C., Natural Red at 32° to 35°C.

177 (2137)

Some changes in the dying cell.

By ROBERT CHAMBERS.

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By ordinary transmitted light the nucleus of the living cell is an optically homogenous body lying in a cytoplasm which is more or less granular. The optical difference between the nucleus and the cytoplasm is more strikingly shown by dark field illumination where the nucleus appear optically empty, whereas the cytoplasm scintillates with bright spots. The cytoplasm has, therefore, been considered to be distinctly heterogeneous in contrast to the optically homogenous nucleus. That this is not true may be seen in the following experiment:

By means of the centrifuge the cytoplasmic granules of the sea urchin egg can be driven to one side of the egg. On cutting away this part one may obtain an egg fragment consisting of protoplasm which is transparent and optically empty, even when viewed with dark field illumination. This fragment is fully capable of developing. We must, therefore, conclude that the cytoplasm may be as optically structureless as the nucleus. When the cell dies, however, a difference in structure with the dark field illumination becomes at once apparent. Coagulating agents, which are not violent in their reaction, such as gentle heat or weak formalin, make the cytoplasm diffusely milky in appearance, owing to the formation of closely packed and uniformly sized spherules. In the sea urchin egg these globules

are about 1 micron in diameter. In tissue cells they are some-
what smaller. The coagulating nucleus in the dying cell, on
the other hand, gives a different picture. Here also uniformly
sized granules make their appearance but, instead of being
evenly distributed, they collect into granular strands which run
together to form a nuclear network.

Another striking death change is a change in the nature of the
surface layer of the cell. Neutral Red, injected into a living
cell, *e. g.,* amoeba, a ciliated cell or a starfish egg, colors the
cell a rose red, indicating a P_H on the acid side. On the death
of the cell the color changes to orange. In the relatively large
amoeba the Neutral Red may even be precipitated out as yellow
crystals. This color change may be explained as follows:
Death destroys the impermeable surface layer of the cell, upon
which the alkaline environing medium diffuses into the freely
permeable coagulated mass and reverses the color of the Neutral
Red.

Another death change which seems to be peculiarly significant
regarding the reducing ability of the different components of the
cell is shown in the following experiment: When living cells
are placed in a solution of Janus Green the mitochondria in the
cytoplasm stain a beautiful blue while the nucleus remains color-
less. As soon, however, as the cell becomes moribund the dye
penetrates the nucleus but instead of staining it blue it gives to
the nuclear network a lilac and sometimes a distinctly reddish
hue. Janus Green is an oxidation product of diethyl safranin
and can be reduced to the red diethyl safranin. In the healthy
cell the dye cannot enter the nucleus. When the cell becomes
moribund the resistance of the nucleus to the penetration of the
dye is diminished and the reducing action of the nuclear sub-
stance then becomes evident. When the cell is quite dead this
reducing ability is lost and, as more of the dye penetrates, the
red color soon becomes masked so that the nucleus finally colors
a deep blue.

The reducing ability of the cell nucleus is also borne out by
the reaction of the cell to vital methylene blue. In sublethal
doses the readily reducible methylene blue enters the cytoplasm,
where it is reduced to its colorless leuco compound. In lethal
doses it accumulates in the nucleus so that when the dead cells
are exposed to air the nucleus stains more heavily blue than the
cytoplasm.

178 (2138)

The therapeutic value of egg yolk in rickets.

By ALFRED F. HESS.

[From the Department of Pathology, College of Physicians and Surgeons, New York City.]

In view of its high content of fat-soluble vitamine, and the association of this vitamine with the anti-rachitic factor, it seemed worth while to test the value of yolk of egg in relation to the prevention and cure of rickets. Mellanby added this to his experimental diet in one instance and was able to cure rickets in a dog. In our experiments young rats were used and the Sherman-Pappenheimer low phosphorus diet was fed. The yolk was given to each animal separately. Table I shows the

TABLE I. PROPHYLACTIC TREATMENT OF RATS WITH EGG YOLK

Weight (g.) at onset and after 28 days	Rickets-producing diet	Daily amount of egg yolk	Radiographic rickets	Path. rickets		Blood P. (mg. per cent.)
				Gross	Microscopic	
50-44	No. 84		marked	R.	R.	2.5
40-44	(High Ca		marked	R.	R.	
50-38	Low P.)		slight	R.	R.	
50-50	No. 84	0.25 g.	neg.	neg.	neg.	4.0
40-40	(High Ca		neg.	neg.	neg.	
50-60	Low P.)		neg.	neg.	neg.	
40-50			neg.	neg.	neg.	
30-66	No. 84	0.33 g.	neg.	neg.	neg.	4.5
40-60	(High Ca		neg.	neg.	neg.	
50-58	Low P.)		neg.	neg.		
40-58			neg.	neg.		
30-60	No. 84	0.5 g.	neg.	neg.	neg.	
40-70	(High Ca		neg.	neg.	neg.	5.0
40-60	Low P.)		neg.	neg.	neg.	
40-70			neg.	neg.	neg.	5.0

result of prophylactic treatment. It will be noted that animals receiving as little as 0.25 g. daily of yolk failed to develop rickets. The yolks contained 450 mg. per cent. of phosphorus so that the protective action cannot be attributed simply to an addition of phosphorus. The gains in weight of these rats were remarkably

good, and their bones were found to be exceptionally well calci-
fied. The addition of white of egg, on the other hand, increased
rather than prevented the development of rickets.

Curative experiments likewise were carried out on rats. In
these tests rickets was induced by the above diet, or by the 5
per cent. dry milk diet, and later supplemented by 1.0 or 0.5 g.
of yolk of egg. After a period of 8 days calcification could be
noted in radiographs. The inorganic phosphate of the blood
also was higher than in the control rats which had not received
the supplementary food.

Similar prophylactic and curative treatment were employed
on infants. Table II summarizes the preventive treatment in

TABLE II.   PROPHYLACTIC TREATMENT OF INFANTS WITH
EGG YOLK

Case	Age (mos.)	Weight (lbs.)	Egg yolk begun	Rickets X-ray (March)	Rickets Clinical (March)	P. (Feb. and	Ca Mar.)
1	12	19½	12-14-22	neg.	neg.	3.8	11.0
2	10	18	1-16-23	neg.	neg.	3.7	12.4
3	14	20¼	1- 3-23	neg.	neg.	4.6	10.8
4	11	18	12-24-22	neg.	neg.	3.9	12.0
5	6½	16½	12-22-22	neg.	neg.	4.5	
6	6½	12¼	12-14-22	neg.	neg.	4.5	11.2
7	12½	16¾	12-14-22	neg.	neg.	4.1	11.8
8	10	17	12-14-22	neg.	neg.	4.3	12.0
9	12	17¼	12-14-22	neg.	neg.	4.0	12.0
10	13½	18¾	12-14-22	neg.	neg.	4.0	
11	10	15¾	1- 8-23	neg.	neg.	4.3	
12	13	25	12-20-22	neg.	neg.	4.0	11.4

twelve cases. It shows that rickets did not develop in any in-
stance, and that the percentage of inorganic phosphate in the
blood during February and March was maintained at the high
level characteristic of the summer months. Curative treatment
was found to be of value, but less potent than cod liver oil.

It is concluded that egg yolks possesses marked anti-rachitic
properties for animals and for infants, far more than any other
natural food stuff. It is very well tolerated and can be recom-
mended as a supplement to the dietary of even very young in-
fants, much as orange juice is used to protect against scurvy.
The yolk has also curative value but definitely less than cod
liver oil.

179 (2139)

The influence of nutrition during the pre-experimental period on the development of rickets in rats.

By ALFRED F. HESS, M. WEINSTOCK and E. TOLSTOI.

[*From the Department of Pathology, College of Physicians and Surgeons, New York City.*]

Whether or not rickets develops on standard "rickets-producing' dietaries may depend on the stock of rats which is used. In our experiments rats from six different sources were tested. It was found that four of these stocks regularly developed rickets when fed from the age of four to eight weeks on the Sherman-Pappenheimer diet; one stock developed rickets but showed a definite tendency to spontaneous calcification of the bones, and one absolutely failed to develop rickets. This refractory group comprised 50 rats four weeks of age. They failed to develop rickets either on the low phosphorus and high calcium diet (No. 84) or on the low calcium and high phosphorus diet (No. 85 C). On the dietary employed by McCollum and his associates, which contains 3 per cent. of calcium carbonate, rachitic lesions developed in some animals but not in others. It is evident, therefore, that the term "rickets-producing dietary" cannot be applied unreservedly but rather in relation to definite stocks of animals.

The divergence in susceptibility is, to some extent, associated with a variability in the percentage of inorganic phosphate of the blood. There is, however, no strict parallelism in this respect. The blood of rats four weeks of age has been found to vary in this constituent from 6.5 mg. to 12.0 mg. per cent.; the calcium has ranged from 6.1 mg. to 8.2 mg. per cent.. The refractory rats had the highest percentage of inorganic phosphate in the blood.

The resistance of this stock is not to be attributed mainly to peculiarity of strain or breed but to previous diet, for it was overcome by modification of the dietary. Pregnant rats of this strain were obtained and fed the stock laboratory dietary as soon as they had given birth to young and throughout the lactating period. At the end of four weeks the young were placed on the

low phosphorus "rickets-producing" diet. It was found that these rats were not refractory but developed rickets to the same extent as the five other stocks that had been tested. Evidently the alteration of the diet during the first four weeks of life was the determining feature. It will be noted that the diet throughout pregnancy was unchanged.

After about the tenth day of life young rats not only suckle but consume supplementary food. The acquired susceptibility may, therefore, have resulted from an inadequacy of the food consumed directly by the young, and not from an inadequacy of the mother's milk. Experiments seem to confirm this point of view. For example, a mother rat which was given 20 drops of cod liver oil daily in addition to our laboratory diet, gave birth to young which were not refractory to rickets. This would lead us to believe that it is difficult to furnish the young with an adequate amount of protective substance through the mother's milk. It seems certain that in young rats the character of the food during the first four weeks of life is of decisive importance regarding their later susceptibility to rickets. This probably indicates that protective anti-rachitic substances can be stored in the body. A discussion of the constitution of the various dietaries used in these tests is deferred until further experiments are carried out.

180 (2140)

The various forms of phosphoric acid in the blood. Findings in rickets.

By T. F. ZUCKER and MARGARET GUTMAN.

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It is well known, that in rickets the inorganic phosphate in the blood is low. We have shown previously that in rachitic as well as in normal blood the inorganic phosphate is the same in the cells and in the plasma, and we have also given evidence

to show that there are three types of acid soluble phosphorus in mammalian blood, namely: inorganic, an organic phosphoric acid compound readily hydrolyzed in neutral or slightly acid solutions, and lastly the "nonhydrolysable phosphate," which cannot be broken down by boiling four hours in dilute acids, but can be determined as inorganic phosphate only after digestion with concentrated nitric and sulfuric acid. In order to complete the blood picture of rickets, we have done complete analyses of the acid soluble phosphorus according to the above scheme on rachitic and non-rachitic children. The results are shown in Tables I and II below.

Here we see that the total acid soluble phosphorus is not lower in rachitic than in normal children, except where the rickets is complicated by anemia. In these cases we would naturally expect a low total acid soluble phosphate since it has been known for some time that by far the greater part of the phosphorus of the blood occurs in the red cells. In anemia, however, the inorganic phosphate is not low.

In rachitic children the inorganic phosphate is not only lower in actual amount than the normal; (2.7 mg. against 4.6 mg.) but its percentage of the total is also lower. (13 per cent. against 22 per cent.).

The non-hydrolysable is increased above the normal (57.8 per cent. against the normal 48.5 per cent.).

Similar analyses were done on rachitic and non-rachitic rats (Table III). Here again we find in the rachitic animals a lowered inorganic phosphate (2.9 mg. or 15 per cent. as opposed to 6.2 mg. or 27 per cent. of the total acid soluble), and the increase of the non-hydrolysable. 60.7 per cent. against 42 per cent. in the normal). Owing to the fact that the blood phosphorus in rats can easily be influenced by the phosphorus level of the diet (not the case in human beings) the total acid soluble phosphate does not show the same constancy.

The significant facts shown by our data are:

1. In rickets the total acid soluble phosphate does not fall below the normal range.

2. The inorganic phosphate is reduced in rickets by an increase of the non-hydrolysable, the organic hydrolysable remaining the same.

3. In anemic blood the total acid soluble phosphate may be low without affecting the inorganic phosphate.

TABLE I.   DISTRIBUTION OF PHOSPHORIC ACID IN RACHITIC BLOOD

Name	Age	Inorganic mg.	Inorganic %	Total acid sol. mg.	After boiling mg.	Org. mg.	Hydrolysable %	Non-Hydrolysable mg.	Non-Hydrolysable %	
X. G.	17 mo.	2.9	11.6	25.0	10.6	7.7	30.8	14.4	57.6	
W. R.	10 mo.	2.5	10.4	22.0	10.0	7.5	34.0	13.0	54.5	
W. M.	7 mo.	2.9	14.2	21.1	9.3	6.4	30.5	12.3	57.0	
E. G.	13 mo.	3.1	15.6	19.9	8.7	5.6	28.1	11.2	56.5	
H. C.	15 mo.	3.4	15.2	21.7	8.7	5.3	24.5	13.0	60.0	
J. M.	6 mo.	2.5	11.9	21.0	9.7	7.2	34.3	11.3	54.0	
M. B.	12 mo.	2.8	10.0	28.6	12.3	9.5	33.2	16.3	57.0	
A. G.	15 mo.	2.4	14.0	17.2	6.7	4.3	25.0	10.5	61.0	
L. M.	8 mo.	2.2	12.5	17.6	7.4	5.2	29.6	10.2	58.0	
Aver.		2.7	13.0	21.5			30.0		57.8	

RICKETS WITH ANEMIA

Name	Age	Inorganic mg.	Inorganic %	Total acid sol. mg.	After boiling mg.	Org. mg.	Hydrolysable %	Non-Hydrolysable mg.	Non-Hydrolysable %	
S. Sc.	15 mo.	2.3	15.4%	14.9	5.7	3.4	23.0	9.2	62.0	cell volume 18%
S. Sc.	16 mo.	2.7	15.5%	17.4	8.5	5.8	33.3	9.0	51.5	cell volume 26%
S. H.	9 mo.	1.6	10.%	16.0	6.8	5.2	32.5	9.2	57.6	anemia

TABLE II.   DISTRIBUTION OF PHOSPHORIC ACID IN NON-RACHITIC BLOOD

Name	Age	Inorganic mg.	Inorganic %	Total acid sol. mg.	After boiling mg.	Org. mg.	Hydrolysable %	Non-Hydrolysable mg.	Non-Hydrolysable %	
J. R.	14 mo.	4.7	27.0	17.5	8.0	3.25	18.5	9.5	53.0	
X. Ro.	1 mo.	4.2	15.8	26.6	13.3	9.1	34.1	13.3	50.0	
L. F.	23 mo.	4.1	19.5	21.0	11.0	6.9	32.9	10.0	47.6	
M. D.	6 yrs.	4.7	19.7	23.8	12.5	7.7	32.4	11.5	46.0	
J. H.	?	4.1	19.0	21.6	11.0	6.8	31.6	10.6	49.0	
J. T.	8 mo.	4.8	23.2	20.5	10.5	5.7	28.0	10.0	49.0	
R. M.	7 yrs.	4.7	21.8	21.8	11.8	7.0	32.1	10.0	47.1	
1. P.	18 mo.	5.0	21.3	23.5	13.0	8.0	34.0	10.5	44.5	
N. T.	?	5.0	22.0	22.8	11.6	6.6	28.9	11.2	51.0	
W. C.	4 yrs.	5.0	22.5	22.2	11.1	6.1	28.8	11.1	50.	
W. A.	15 mo.	5.2	19.8	26.3	12.6	7.4	29.2	13.7	52.0	
M. M.	8 yrs.	4.2	23.0	18.2	11.0	6.0	33.0	7.2	40.	
H. K.	7 yrs.	4.6	19.5	23.6	11.6	7.0	29.6	12.0	51.	
Aver.		4.6	22.0	22.2			30.3		48.5	

WITH ANEMIA

Name	Age	Inorganic mg.	Inorganic %	Total acid sol. mg.	After boiling mg.	Org. mg.	Hydrolysable %	Non-Hydrolysable mg.	Non-Hydrolysable %	
M. Mag.	2½ yrs.	4.0	32.0	12.5	7.7	3.7	29.6	4.8	38.2	cell volume 14%

TABLE III.   DISTRIBUTION OF PHOSPHORIC ACID IN RAT BLOOD
NON-RACHITIC

Diet No.	Total acid sol. mg.	Inorganic mg.	%	Org. Hydro-lysable mg.	%	Non-Hydro-lysable mg.	%
303D	23.0	6.0	26	8.3	36	8.7	38
303	20.6	5.4	26	7.6	37	7.6	37
303A	18.7	4.3	23	6.0	32	8.4	45
302C	22.3	6.1	27	5.3	24	10.8	48
302B	22.6	6.2	27	5.4	24	10.9	48
302A	20.0	6.7	33	6.7	34	6.6	33
302	19.5	7.6	39	5.9	30	6.0	31
301E	28.0	5.9	21	7.5	27	14.6	52
301	30.0	8.0	27	7.6	25	14.4	48
Aver.	22.7	6.2	27		30		42

RACHITIC

Diet No.	Total acid sol. mg.	Inorganic mg.	%	Org. Hydro-lysable mg.	%	Non-Hydro-lysable mg.	%
303C	17.5	2.4	14	4.3	25	10.8	62
303B	17.4	2.7	15	6.4	37	8.3	48
302D	18.7	2.6	14	4.1	22	11.9	64
301A	20.0	2.6	13	3.7	18	13.7	69
301B	20.0	3.6	18	4.0	20	12.2	61
301C	19.2	3.1	16	4.0	21	12.0	63
301D	17.1	3.4	20	3.8	22	9.9	58
Aver.	18.6	2.9	15		24		60

The nature of these diets will be discussed elsewhere. In this table they are simply grouped as rachitic and non-rachitic, according to the X-ray and histological findings.

181 (2141)

Observations on the distribution of anti-rachitic substances.

By T. F. ZUCKER and MARION BARNETT.

[*From the Pathology Department of the College of Physicians and Surgeons, Columbia University, New York City.*]

When Mellanby found that the rickets produced in dogs was prevented or cured by cod liver oil, he thought that since cod liver oid is extremely rich in the fat-soluble A vitamin, that this substance was responsible for the cure and that the deficiency of

fat-soluble A is the cause of rickets. This assumption was soon proved to be erroneous, and a second natural assumption was made by McCollum that the deficiency of another vitamin was responsible for rickets production. No one doubts the existence in cod liver oil of a substance having a favorable influence on mineral metabolism. If this substance is concerned in the rickets of infants then we must be able to show that a normal diet for infants contains the substance in sufficient amount and that when rickets occurs the substance is deficient. It is perfectly well known, however, that rickets occurs quite freely in infants on mother's milk or on best grades of fresh cow's milk. It is, furthermore, a well established fact that rachitic infants are not cured by the addition of liberal amounts of cream to their diet.

Both Park and Howland believe that an anti-rachitic vitamin occurs in foods probably associated with the fat-soluble A in green leaves. Having found a method by which we can materially concentrate the active substance from cod liver oil, we investigated the occurrence of this material in various plant and animal substances. We have subjected the following materials to the processes similar to those by which we obtained a substance from cod liver oil one thousand times as active as cod liver oil itself: butter, cocoanut oil, spinach, carrots, pig's liver and sheep's adrenals. From none of these materials were we able to obtain an extract which even without dilution approached the action of cod liver oil, while from cod liver oil itself we obtained extracts at least one hundred and later more than one thousand times more active than the original cod liver oil. We cannot say that these preparations were entirely free from anti-rachitic substance, but the amounts, if there was any, were so small that they gave minimal and irregular results. Taking as an example the plant materials spinach and carrots—these were treated first with acetone, then with ether, then with alcohol and again ether. We believe that this gave us a thorough extraction of the fats contained in the plant tissues. This fat was then saponified and put through procedures previously described. The extraction of the fats must have concentrated the material at least ten times and the further processes at least one hundred times. On this basis, spinach or carrots if they contain any anti-rachitic material will require four kilogram to furnish as much as one teaspoonful of cod liver oil. We can safely say then that

by means of a method which can reasonably be expected to give results, we have been unable to confirm the assumption that green leaves and a number of other food materials contain a significant amount of anti-rachitic substance.

How then are we to explain the results of McCollum and others that butter and cocoanut oil produce partial cures. The following experiment, we think, throws light on the subject: When rats are kept for four weeks on a diet of flour and a salt mixture containing 2.9 per cent. of calcium lactate, rickets is regularly produced. When we use this diet with the addition of 20 per cent. of either cotton seed oil or crisco, a hydrogenated product from cotton seed oil, rickets is still produced. If, however, we lower the percentage of calcium lactate to 1.5 per cent., the diet without fat produces rickets, while the addition of 20 per cent. of crisco entirely prevents it and the cotton seed oil does not prevent it but merely gives a somewhat lesser degree of rickets. In this case vitamin action is excluded, but we find that the hydrogenated product prevents rickets. The hydrogenation has increased the melting point of the oil; and the soaps from crisco are more insoluble than those from cotton seed oil. Our interpretation is, that in the case of crisco more insoluble calcium soaps are formed in the intestines and by precipitating calcium soaps phosphate in soluble form which would otherwise be precipitated as calcium phosphate is made available for absorption. With a great excess of calcium in the diet containing 2.9 per cent. of calcium lactate, the precipitating action of crisco is, however, insufficient to prevent rickets. Now if we turn back to cocoanut oil, we can easily see how this fat whose soaps are notoriously insoluble will show such a marked rickets preventing action.

We also see from this experiment that the rickets producing qualities of a diet depend on the exact adjustment of quite a number of factors, and we are led to believe that the slight rickets curing action of butter recorded by McCollum is due to fatty acids in the butter rather than a vitamin, particularly since we are unable to obtain any concentrated active substance from butter, and since after all rickets in infants cannot be prevented or cured by either butter or cream.

While we cannot say that we have proved the non-existence of an anti-rachitic vitamin the facts adduced certainly detract

from the plausibility of the assumption that there is one. Until the presence of such a vitamin is established in natural infants' diets in cases where rickets is prevented and a deficiency in diets of cases where rickets occurs it will be safer to approach the subject with an open mind. The rickets-curing substances in cod liver oil and in egg yolk might just as well be looked upon as therapeutic agents, possibly internal secretions, which will prevent or cure rickets.

182 (2142)

On the nature of pneumococcus antigen.

By W. A. PERLZWEIG and G. I. STEFFEN (by invitation.)

[*From the Hygienic Laboratory of the U. S. Public Health Service and the Second Medical (Cornell) Division and the Pathological Department of Bellevue Hospital, New York City.*]

Various extracts of killed pneumococci have been studied for the purpose of determining their immunizing power and their freedom from toxic products. A non-toxic pneumococcus antigen of high potency can be made by suspending the sediment from a centrifuged pneumococcus broth culture in saline and digesting the pneumococci with 0.2 per cent. trypsin for 24 to 48 hours. The undigested portion is centrifuged off and the metaproteins in the supernatant fluid precipitated with acid. After filtration the filtrate is thrown into 7 volumes of 95 per cent. alcohol, the resulting precipitate filtered off and the alcoholic solution evaporated in vacuum. The residue in the flask is taken up in saline and made up to the original volume of the saline suspension. This solution contains the immunizing antigen of the pneumococcus. Mice which had received three subcutaneous injections of this antigen were protected against a hundred thousand lethal doses of a pneumococcus broth culture, injected intraperitoneally.

Human volunteers were injected subcutaneously with the alcohol soluble fractions of pneumococcus Type I, II, and III.

Three doses were given, the total corresponding to 50 to 75 billion of killed pneumococci. Practically no local, and absolutely no general reaction was observed after any of the injections. Following the injection of antigen protective substances against the three fixed types of pneumococcus could be demonstrated in the serum of the volunteers.

The pneumococcus antigen is not adsorbed by Lloyd's reagent, and does not diffuse through a collodion membrane.

Saline suspensions of pneumococcus Type I which had been stored in the refrigerator for several months were shown to contain a large amount of the antigen in the supernatant fluid. Mice were injected subcutaneously with two or three doses of the supernatant fluid which protected them against a hundred thousand lethal doses of a broth culture of pneumococcus Type I, injected intraperitoneally. Some of the immunizing antigen is retained by the killed pneumococci.

A chemical analysis of the supernatant fluid from old pneumococcus vaccine gave the following result: Total nitrogen, 13 mg. per 100 c.c., non-protein nitrogen, 12.9 mg. per 100 c.c., of which 8 mg. were in the form of amino acid nitrogen. Phosphate present. Reaction P_H 7.8. Biuret, Millon, Xanthoproteic and Hopkins-Cole reaction were all negative. Precipitation tests with heat and acid, sulphosalicylic acid, potassium ferrocyanide and acetic acid were all negative. A slight turbidity is produced by 9 volumes of 95 per cent. alcohol, trichloracetic acid, conc. hydrochloric and nitric acid. The precipitate produced by 9 volumes of alcohol was found to be chiefly phosphates.

The filtrate from old pneumococcus vaccine gives clear cut precipitin reaction with homologous antiserum in antigen dilutions up to 1:120.

The immunizing antigen of the pneumococcus and the precipitinogen appear to be two distinct entities, as we have encountered some pneumococcus antigen solutions which fail to give precipitin reaction with homologous antiserum, but produce immunity in mice when injected subcutaneously.

183 (2143)

Indican as influenced by bacillus acidophilus therapy.

By LILLIAN SEGAL KOPELOFF and NICHOLAS KOPELOFF.

[*From the Psychiatric Institute, Ward's Island, N. Y.*]

Over 150 urinanalyses were made on 40 psychotic patients. The number of daily bowel movements were recorded, and showed a range varying from normal to severe chronic constipation. Various experimental procedures with *B. acidophilus* were employed. Such an investigation must be continued for a considerable period of time before any conclusions can be drawn. However, in reporting progress, it is interesting to note that in only five instances did the indican present exceed 20 mg. which is generally regarded as the upper limit of normal excretion. Some of the patients with severest constipation had very small amounts of indican present in the urine. In general patients who received *B. acidophilus* milk had a smaller amount of urinary indican than patients receiving *B. acidophilus* milk in which the viable organism had been killed.

184 (2144)

Leucocytes in relation to the mechanism of thyroid-accelerated metamorphosis in the larval frog.

By H. E. JORDAN and C. C. SPEIDEL.

[*From the Department of Histology and Embryology, University of Virginia Medical School University, Virginia.*]

In the larval frog the chief locus of blood-cell formation is the kidney. The intertubular regions of this organ are filled with many erythrocytes and leukocytes in various stages of development. The spleen and, to a less extent, the mesentery are also hemopoietically active, but are of minor importance. In the adult frog, however, conditions are different. The spleen is

here the principal hemopoietic center, the kidney playing little part. At some time in the developmental history of the frog, therefore, the chief locus of blood-cell formation is shifted from the kidney to the spleen. A study has been made to determine the effect upon the hemopoietic centers of accelerated metamorphosis induced by feeding tadpoles with thyroid extract.

A few days after the first administration of thyroid extract definite changes may be seen in the kidney and spleen. The intertubular stroma of the kidney is largely drained of its blood-cells. Some stimulation of the hemopoietic tissue that is left occurs. Erythrocytes, lymphocytes, special granulocytes (pseudo-eosinophils or neutrophils) and eosinophils are drawn from the kidney in great numbers. A marked shrinkage in size of this organ results which is evident microscopically. At the same time a myeloid metaplasia is effected in the spleen and mesentery. Erythrocytes are drained from the spleen and the splenic lymphocytes stimulated to differentiate into other erythrocytes. Lymphocytes from other parts of the body migrate to the spleen and are incorporated in it. The net result of this activity in animals in which metamorphosis is not too greatly accelerated is an increase in the proportion of lymphocytes to erythrocytes, and some increase in absolute size of the spleen. In these animals it may be said that the partial drainage of the intertubular regions of the kidney, and the stimulation of the spleen, apparently indicate the initiation of the shift of the main hemopoietic locus from the kidney to the spleen. Whether or not this shift can be carried to completion experimentally has not yet been determined.

Accelerated metamorphosis results in high mortality. All of our thyroid-treated tadpoles have died before complete resorption of the tail. Death usually occurs in this condition of metamorphic stasis. Examination of the hemopoietic organs of animals that have reached this stage suggests that death comes as a result of anemia. The spleen and kidney show that great numbers of their blood-cells have been drawn from them, the spleen being often totally exhausted. Accelerated metamorphosis sets up a demand for erythrocytes to furnish the basis for increased oxidation, and for leukocytes to aid in the processes of regressive and progressive change. The lymphocyte is the progenitor of the erythrocyte and of various types of leukocytes, and is itself derived from the mesenchymal cell. The successful prog-

ress of accelerated metamorphosis depends largely upon the ability of the lymphocyte to supply the erythrocytes and leukocytes needed. Metamorphic stasis and death we interpret to mean the failure of the lymphocyte to meet the extraordinarily great hemopoietic demands set up by the chain of reactions following repeated thyroid administration. This interpretation agrees with our observations on adult frogs following experimental hemorrhage and injection of a hemolytic toxin.

In a recent series of experiments on thyroid-treated tadpoles Swingle[1] has attempted to carry them over the stage of metamorphic stasis by means of hypophysis grafts. He finds the response variable. In some cases this treatment aids the progress of metamorphosis, in others it does not. We suggest that this variable response may be correlated with the hemopoietic reserve of the animals. In those in which the lymphocytes are still present in sufficient numbers the hypophysis graft may serve to aid metamorphosis. In those in which the lymphocytes have been practically exhausted the graft may perhaps have no effect.

Microscopic examination of several regions of a tadpole in process of metamorphosis reveals that there is a definite differential distribution of leukocytes. In the tail, a region of regressive change, the predominating types of leukocytes are lymphoid phagocytes (phagocytes derived from lymphocytes) and special granulocytes. Lymphocytes and eosinophils occur only in small numbers. In the intestine, also a region of regressive change, the predominating types are eosinophils and lymphoid phagocytes. The chief function of the eosinophils and special granulocytes in these regions appears to be to give off substances which help to break down adjacent tissues, thus rendering these more easy of ingestion and phagocytosis by the phagocytic leukocytes (lymphoid phagocytes chiefly). The eosinophils and special granulocytes themselves often go to pieces in the process and may then be ingested by the phagocytes.

In the rapidly growing limbs, regions of progressive change, the predominant type of leukocyte is the lymphocyte. Some lymphocytes are brought in by the blood-vessels, but the great majority are differentiated in situ from the mesenchyme. A conspicuous lymphocyte layer is present immediately beneath the basement membrane of the epidermis. Eosinophils, special

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[1] *Journ. Exp. Zool.*, 1923, xxxvii, 219-257.

granulocytes and phagocytes are almost entirely absent. Carrel's recent experiments[2] dealing with the effect of leukocyte extract and leukocyte secretions upon tissue cultures show that leukocytes give off substances that stimulate growth of surrounding cells. Carrel used a mixture of all leukocytes in these experiments. In the rapidly growing tadpole limb, since the lymphocyte is the only type of leukocyte present in any numbers, it is obvious that any growth-promoting leukocytic secretion must come from it. Examination of the skin from the abdomen and back of the same tadpoles that have the conspicuous lymphocyte layer in the limbs shows that there is no similar collection of differentiating lymphocytes in these regions (regions in which little or no growth is taking place). From the negative standpoint, it would seem that eosinophils, special granulocytes and phagocytes could be ruled out from having a possible growth-promoting function since they predominate in the tadpole in regions of regressive change. We suggest, therefore, that the growth-promoting function of leukocytes, as demonstrated by Carrel, should probably be ascribed to the lymphocyte component of leukocytes.

In conclusion, it may be pointed out that the leukocytes are an important part of the mechanism by which the thyroid secretion brings about its characteristic results. Metamorphic differentiation is largely a sequence of progressive and regressive changes in various parts of the body. Lymphocytes are correlated with the former, granulocytes and phagocytes with the latter. The successful maintenance of the increased oxidation following thyroid administration depends upon the lymphocyte as progenitor of the erythrocyte.

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[2] *Journ. Exp. Med.*, 1922, xxvi, 385-391.

185 (2145)

New quantitative observations on the penetration of acids and alkali bicarbonates into living and dead cells.

By M. M. BROOKS (by invitation).[1]

[*From the Division of Pharmacology, Hygienic Laboratory, Washington, D. C.*]

Cells of the green alga *Valonia* from which cell-sap can be obtained free from contamination in sufficient quantities for accurate observation were placed in solutions of acids dissolved in sea water. The concentrations of acid were such so as to give a P_H of 3.6. At intervals cells were taken out and the H ion concentration of the sap noted. It was found that two sets of observation were necessary; one set comprised the H ion concentrations of the sap as it came from the cell and containing considerable free CO_2; the other when the free CO_2 was removed by thorough aeration by means of a stream of CO_2-free air. This method showed that normal cells have a P_H of 6.2 to 6.4 when CO_2 is present and 6.6 to 6.8 when CO_2 has been removed. The acids used could be divided into two broad classes with respect to their action on the cells: to the first class belong HCl, HNO_3, H_2SO_4, arsenic, phosphoric, tartaric, citric, oxalic, mono- di- and tri-chloracetic acids. To the second class belong acetic, butyric, benzoic and salicylic acids. All the acids of the first class increased the amount of free CO_2 in the cell-sap, presumably by decomposing the bicarbonates present. When the P_H of the cell-sap had reached 5.2 it remained stationary until no more CO_2 could be detected by the method, the time varying according to the acid used. Acids of the second class penetrated rapidly and produced little or no free CO_2 in the cell-sap. Acids of the first class appear to penetrate less rapidly than they actually do, because instead of existing free in the cell-sap they form neutral salts with the basic ions of the cell bicarbonates. The carbonic acid liberated in this process is so weak as to have relatively little effect on the P_H.

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[1] Published by permission of the Surgeon-General, United States Public Health Service.

Dead cells behave somewhat like living ones. In the case of acids of the first class, free CO_2 could be detected as in living cells: while acids of the second class penetrated dead cells at about the same rate and in apparently the same manner with reference to CO_2 liberation as in living cells.

The rate of penetration of an acid depends therefor upon the nature of the acid used. Previous investigators have neglected the fact that carbonate decomposition would delay changes in the H ion concentration of the cell; and have therefore been misled as to the rates of penetration of acids of the first class.

II

Plants were also placed in sea water containing the following: (1) enough CO_2 to make the P_H 7.2; (2) $KHCO_3$, .03M; (3) $NaHCO_3$, .03M; the P_H of the last two solutions was 7.8. In all three cases free CO_2 accumulated rapidly in the sap, the P_H of which first became about 5.2 but later slowly increased and ultimately exceeded the normal. The P_H of sap from which the free CO_2 had been blown out was observed to have increased; and the longer the cells remained in the solution the more alkaline the CO_2-free sap became until finally a P_H of 8.6 had been reached. This process was quicker in the case of $KHCO_3$ than in that of $NaHCO_3$, possibly because K′ penetrates more rapidly than Na′. The known preponderance of K′ in the cell-sap of *Valonia* may be in part due to such selective permeability. When NaCl, KCl or KNO_3 were dissolved in sea water in the same concentration (.03 M) no such changes in P_H were noted. They are therefore characteristic effects of the penetration of $HCO_3′$ into the cell. Details of this study will appear in a later journal.

186 **(2146)**

The effect of thyroid products on Paramecium.

By LORANDE LOSS WOODRUFF and WILBUR W. SWINGLE.

[*From the Osborn Zoological Laboratory, Yale University, New Haven, Conn.*]

Studies of Nowikoff, Shumway, and Budington and Harvey on the influence of thyroid products on Paramecium have apparently shown a marked increase in the rate of reproduction, and their results have been generally cited as a corroboration of the view that the active principle of the thyroid directly accelerates cell metabolism.[1] Recently Riddle and Torrey have failed to find an increased reproduction in Paramecium following subjection to thyroxin.[2]

We have reinvestigated the influence of thyroid products by a series of experiments on a pedigree race of Paramecium, using the organism as a "biological indicator" and following the general technique employed in other studies on Paramecium in this laboratory.[3]

The results, which will be published in detail elsewhere,[4] show in a clear cut manner that neither thyroxin (Squibb's) nor commercial dessicated thyroid, or fresh dessicated thyroid of the turtle produce any significant acceleration of the division rate of Paramecium.

Data to the contrary published by previous investigators apparently are attributable chiefly to variations in the bacterial food supply which the different media afforded the Paramecia.

Accordingly, all the evidence from studies on Paramecium to the effect that thyroid products accelerate cell anabolism is, we believe, erroneous.

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[1] Nowikoff, M., *Arch. f. Protistenkunde*, 1908, xi; Shumway, W., *Jour. Exper. Zool.*, 1914, xvii; *Ibid.*, 1917, xxii; Budington, R. A., and Harvey, H. F., *Biol. Bull.*, 1915, xxviii.

[2] Riddle, M. C., and Torrey, H. B., *Proc. Amer. Soc. Zool., Anat. Rec.*, 1923, xxiv.

[3] Woodruff, L. L., *Biochem. Bull.*, 1912, i; Woodruff, L. L., and Underhill, F. P., *Jour. Biol. Chem.*, 1913, xv.

[4] *Journal of Biological Chemistry.*

187 (2147)

The effect of ultra-violet rays on rats in the circular maze.

By DAVID I. MACHT and ELMER J. TEAGARDEN, JR.

[*From the Pharmacological Laboratory, Johns Hopkins University, Baltimore, Md.*]

The present research was undertaken in connection with the effect of light on the toxicity of quinine and quinidine sulphates for rats trained in the circular maze. In order to study the effects of the drugs it was essential to learn the effects of ultra violet light itself on the animals. The method employed was the same repeatedly described by Macht and his collaborators in various papers. White rats were trained in the circular maze so as to find their way from the entrance to the center without committing any errors and in the shortest period of time possible. After this preliminary training the animals were exposed to the radiations of the Hanovia Alpine Sun Lamp for periods varying from 10 to 15 minutes or longer and the subsequent behavior of the animals was again studied.

Six young adult male rats, age about six months, and three old rats, age about one year, were employed. Thirty experiments in all were made on the young rats and twenty-four experiments on the older animals. A careful analysis of all the data obtained before and after radiation indicated that the effect of ultra violet rays on their behavior in the maze is either negative or more often, slightly stimulating. This stimulating effect was manifested by more rapid muscular activity and persisted for several hours after radiation. All of the animals were found to have resumed their original normal condition within twenty-four hours. It is possible that the increased muscular activity or stimulation is merely the result of irritation, on the other hand no other deleterious effects were produced in the animals, even conjunctivitis occurred only occasionally and was never marked. The general condition of the animals, their nutrition and behavior were not found to be impaired in any way.

188 (2148)

Hereditary factors in body build.

By C. B. DAVENPORT.

[From the Station for Experimental Evolution, Cold Spring Harbor, N. Y.]

Some physiologists of nutrition seem inclined to conclude that all obesity is exogenous or nutritional obesity; that any excess of energy of intake over what is required to maintain the temperature of the body, and to enable it to do its work, due allowance being made for loss in feces and urine, must be stored as body fat. That there are endogenous factors is shown in cases of hypothyroidism and disfunction of some other endocrine glands. But Van Noorden, for example, is inclined to reject heredity as a factor in obesity. It is, indeed, recognized that in some races and in some families the individuals have heavier body build than in others. Thus the Scotch are slender and the Greeks and Eastern Jews are stout. But the racial as well as the familial idiosyncracies in build he would explain on the grounds of what may be called social heredity *i. e.*, the handing on of traditions of feeding. On the other hand the degree of functioning of the endocrine glands is hereditary.

To see if there are hereditary factors in build, a mass of between 2,000 and 3,000 sets of measurements of build, taken chiefly from random family records was distributed by the indices of build, and the number of individuals possessing each index was determined. This gives Figure 1. Figure 1 is the distribution of build of adults referred to about 50 years of age. These adults are grouped in five large classes: Very Slender, Slender, Medium, Fleshy and Very Fleshy. A similar polygon might be made persons 18 years of age, 14 years, 12 years, 8, 4, and 1 year or at birth. The means at these different years would vary —thus from 55 per cent. relative chest at 50 years to 47 per cent. at 12 years and to 67 per cent. at birth.

A figure was shown of the curve of average relative chest girth from birth to 21 years. There is an ontogenetic change in mean build and, at any age, a variability in build.

Again the variability polygon differs in shape and position in different families—in the progeny of different matings. Also the variability is different with different distribution polygons. What does this mean? Is it fully explained by social heredity?
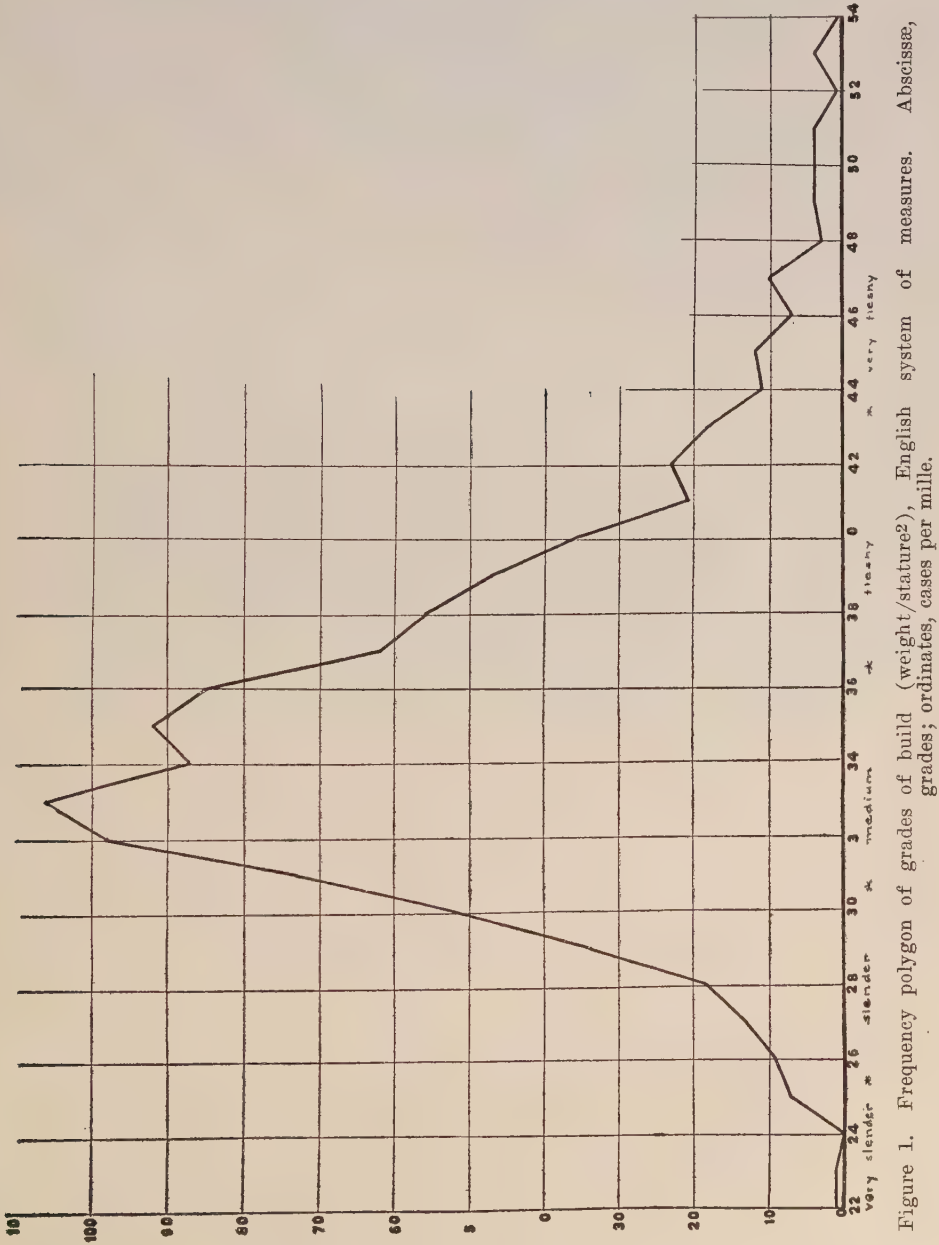
Figure 1. Frequency polygon of grades of build (weight/stature²), English system of measures. Abscissæ, grades; ordinates, cases per mille.

No; for one outstanding fact is that two healthy brothers may differ at the same age, one being slender, the other very fleshy. Different human strains differ in build just as Jersey steers differ from Aberdeen Angus steers. Armsby has inquired into the cause of the difference in build of such steers: he concluded that it is partly due to different amounts of food consumed; the Jersey steer is a lighter eater. It is also due to the fact that the Jersey's excess calories are used in building up protein which stores up a relatively great amount of energy, per kilogram, while in the Angus, the excess calories are stored in the form of fat which uses up relatively little energy per kilo. There is a difference in the method of metabolizing. Apparently this difference is found also in families,—so that we have some families in which the members store fat, in others, protein; at least, some fatten easily, others with difficulty. Probably the constitutional difference in human families is that which distinguishes chow dogs and grey hounds, Cochin china pigs and razor backs.

Returning to humans, one finds that the offspring of two very slender parents are practically all slender. But the progeny of two parents of medium build in certain cases range from very slender to very fleshy. Slenderness is recessive; but fleshiness is not differentiated genetically from slenderness by a single factor, but sometimes by at least two independent factors, possibly more. However this may be, the analysis that can be applied to the 500 matings studied, shows that the capacity of fattening easily depends on germinal factors just as truly as stature does.

189 (2149)

The action of salicylates on the isolated heart.

By WILLIAM SALANT and ROBERT L. JOHNSON.
(With the assistance of MISS SALLIE RUTLEDGE).

[*From the Laboratory of the Department of Physiology and Pharmacology, University of Georgia, Augusta, Georgia.*]

Most of the experiments were carried out on the frog heart but turtles were also used occasionally. Sodium salicylate in different concentrations in Ringer's solution produced the following results:

A solution of 1:2000 caused no effect or stimulation. When the concentration was 1:1000 and the heart was exposed to the action of the salicylate for ten to fifteen minutes considerable depression occurred. Slight improvement was sometimes noticed when the sodium salicylate was discontinued, but complete recovery was never observed. Stronger solutions produced still greater depression; 1:500 caused cessation of heart action in two minutes, but some improvement also occurred in this case when perfusion with Ringer's solution was resumed. With a concentration of 1:250 depression was still more pronounced.

Marked depression of the heart also occurred when it was perfused with very weak solutions of acetylsalicylic acid (1:4000 to 1:2000). It was found, however, that this was due to the increased hydrogen ion concentration of the solution, for by adding sodium hydroxide or sodium bicarbonate until the P_H was 7.4 or 7.5 (the same as that of Ringer's solution) stimulation was produced when the heart was perfused with the acetyl derivative of the same molecular concentration as that of sodium salicylate. Stronger concentration (1:250), however, caused cardiac depression though the hydrogen ion concentration was corrected as above to correspond with that of the Ringer's solution. The results show, therefore, that sodium salicylate is more toxic than the corresponding acetyl derivative. Similar results were obtained by Dreser[1] who perfused the frog heart with sodium salicylate and aspirin in defibrinated ox blood.

Experiments were also performed with methyl and ethyl salicylates. A saturated solution of the former (0.07 per cent. or less) produced complete heart block within two or three minutes which was promptly removed by perfusion with Ringer's solution alone.

Similar effects were produced by a saturated solution of ethyl salicylate, but the arrest of heart action set in after a period of five to ten minutes or longer. Recovery occurred also as after perfusion with methyl salicylate.

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[1] H. Dreser, *Archiv. fur Ges. Physiol.*, 1899, lxxvi, 306.

190 (2150)

A new auditory test apparatus.

By A. G. POHLMAN and F. W. KRANZ.

[*From the St. Louis University and Wallace Clement Sabin Laboratory of Acoustics, Geneva, Illinois.*]

Practically all tests on the location of deafness are made by comparing the acuity of hearing as found by use of the tuning fork by air transmitted sound with that determined by applying the stem of the fork to the head bones. The disadvantages of the tuning fork test are both of a physical and physiological nature. No two forks are alike nor can the activation of any fork be expressed in terms of absolute or even reproducible units. It is also quite impossible to establish the threshold of audition either by the distance through which a fork is heard or in the case of bone transmission, by the length of time a given fork is heard when applied to the head. Scientific work on the problem of auditory acuity demands first of all that a standard method of measurement of acuity by air transmission be adopted. It is also essential that a new type of apparatus be devised which shall replace the tuning fork for the determination of minimum audibility for bone transmitted sound. Such an apparatus must fulfill several requirements. It must be possible to deliver the energy to the bone in a series of graded intensities. It must also be so arranged that the stimulus may be exhibited at a given pitch and a given intensity for an indefinite period of time. It must be capable of accurately reproducing any given intensity. Finally, for clinical purposes it must be easily transported, standardized and practically fool-proof.

Such a piece of apparatus has recently been developed at the Riverbank Laboratory. In its refined form, the range of intensities available will be sufficient to test subjects of all grades of acuity of hearing. The clinical type of instrument is portable and may be connected with any lamp socket on the ordinary sixty cycle alternating current. Standardized reproducible intensities may be obtained both for air and bone transmitted sound at 60 and 120 p.p.s., the vibrating elements being actuated electromagnetically. It is hoped that this device will serve the otologist as an accurate instrument for diagnostic purposes in the field in which the low pitch tuning fork is now used.

191 (2151)

Light as a factor in fish dispersal.

By F. E. CHIDESTER.

[*From the West Virginia University, Morgantown, W. Va.*]

During the summer of 1922, certain observations and experiments were made at the Woods Hole Laboratory of the Bureau of Fisheries that add to our ability to interpret the behavior of fishes in the field.

Ten fish at a time, males of the species *Fundulus heteroclitus*, about 9 centimeters in length were placed in each of twin troughs 10 feet x 4½ inches x 4 inches, with glass plates at the ends and with a middle intake at the bottom and the outlet under a glass plate which could be changed in position.

The troughs were conspicuously marked off in feet and records of the position of the fish were taken at intervals of 15 minutes during the course of the experiments. The records were for example—2 P. M. 6 (fish) at 4 (feet from illumination); 4 at 7.

Light adaptation was secured by means of a 200 watt Mazda light suspended above the troughs; while dark adaptations were accomplished by leaving the fish overnight in running water, available in the troughs at all times, or by the use of light proof shades for shorter periods of preliminary adaptation.

Light stimuli were applied by means of two 40 watt Mazda lights of equal intensity, placed in twin lamp houses, which like the troughs, were blackened inside and outside and which were equipped with rectangular slits, ½ inch in width and 2 inches in length, so that light could be directed in a narrow beam through the clear glass ends of the troughs or through the Eastman monochromatic filters that were sometimes interposed.

The experiments to be here recorded are as follows:

I. Temperature uniform, current stopped 15 minutes prior to experiments.

1. Dark adaptation 12 hours (over night), room darkened, light stimulus applied for 10 trials at left and 10 trials at right of trough.

Temperature	Positive heliotropism
20.5°C.	63.3 per cent.
18. °C.	80. per cent.

These experiments serve to indicate that migration in the cooler water may be influenced somewhat by the action of light, and may even be in part due to light. However, the well-known fact that hibernating fish come out and swim around under thin sheets of ice, after the sun has risen, may be directly correlated. It is quite possible that before the sun has warmed the water, its rays may serve to stimulate surface swimming. The clear sky contributes to attractiveness of the shallower waters offshore.

Alewives are known to rest in small pools at night and to travel only by daylight. On the other hand, shad, although they travel by daylight, evince great fear of shadows. Light sensitivity is well marked in many of the migratory fishes, although it is quite variable.

2. Dark adaptation 12 hours, room darkened, monochromatic lights used.

When monochromatic lights were used, the reactions to red, blue and green seemed attributable to intensity rather than to color. The investigations of White, Reeves, Reighard and others will be discussed in another more complete resume of the subject of fish responses.

3. Light adaptation 3 hours, room darkened at time of experiment.

The average percents of positive heliotropism were so remarkably low in the case of both the normal temperature of 20.5°C. and the lower one of 18°C. that the movements were considered as random movements. After one-half hour of darkness, the fish began to react definitely to light stimuli. Since the fish were in their troughs undisturbed by agitation, the distinctly negative results thus obtained may be considered as more reliable than those reported by Hess under exaggerated stimuli from moving the aquaria to the window and then into a dark-room.

II. Temperature of troughs graduated from left to right; fish dark adapted 12 hours, illumination stimuli changed from warmer end to cooler end.

| Temperature | | Positive heliotropism | |
Left	Right	Left illuminated	Right illuminated
32 C.	28 C.	60 per cent.	70.25 per cent.
30 C.	26 C.	65 per cent.	72.5 per cent.
17 C.	21 C.	60 per cent.	75. per cent.

It is evident that when given a choice of temperature and light combinations, the fish used is influenced somewhat by the attractiveness of light, even toward water much warmer than the optimum at the season. It is also evident that responses to light in fish are not remarkably accelerated by water warmed as much as 10 degrees above the normal of 20.5C. at the time these experiments were made.

ABSTRACTS OF COMMUNICATIONS.

Peking Branch.

Second meeting.

Peking, China, March 7, 1923.

192 (2152)

The pharmacology of Tang Kuei.

By B. E. READ and CARL F. SCHMIDT.

[*From the Laboratory of Pharmacology of Peking Union Medical College, Peking, China.*]

Tang Kuei, identified by E. H. Holmes as the root of Angelica Anomala, var. Chinensis, is used in native medicine in the treatment of menstrual and puerperal disorders and sterility in women, being sold as thin slices of a woody root, having a sweetish taste and an aromatic odor. It is on the western market under the name of "Eumenol." Previous investigators[1,2] ascribed its action to volatile ingredients, being unable to isolate from it an alkaloid, glucoside, or other active principle.

A simple extract of the drug, injected intravenously in anesthetized dogs, uniformly caused: (a) marked circulatory depression; (b) prolonged and striking diuresis; (c) contraction of uterine, bladder and intestinal muscle.

After removal of volatile material by distillation, the residue was still effective; the distillate sometimes caused contraction of uterus or gut, but large doses were required.

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1 Buffalini, *Annali di farmacologia*, 1900, 140.

2 Sakai, *Tokyo Igakeekai Zosshi*, 1916, xxx, 19.

The circulatory depression was due to direct action on cardiac muscle; the depressant material was precipitated by mercury. The residue contained sugar, which was removed by glacial acetic acid. From this residue crystals were obtained which were powerful stimulants to smooth muscle of uterus, intestine, and arteries, caused constriction, followed by dilatation of kidney vessels, with slight diuresis and a prolonged rise in blood pressure, from peripheral constriction. In the dog, 0.5 to 5 mg. caused contraction of the uterus, pregnant or non-pregnant; in the rabbit, similar results were obtained with 0.1 to 0.4 mg. Intestinal muscle was affected also, but larger doses were required. Isolated rabbit uterus was strongly stimulated by one part in two millions of this material; strips of human uterus responded to one part in one million; isolated rabbit gut showed an increase in rhythmic movement and in tone, but never a tonic spasm, and the effect was not influenced by atropine; in all these preparations, the effect disappeared on substituting fresh solution, and could be brought out repeatedly by adding more of the drug. The physiologic effects were very similar to those of pituitary extract, but, unlike pituitary, could be brought out repeatedly without weakening or reversal.

The nature of these crystals remains undetermined. They are organic, are non-nitrogenous, non-glucosidal, freely soluble in water, and melt at 52-58°C. We believe that they are responsible for the therapeutic smooth muscle effects of Tang Kuei.

Other constituents of the drug include (a) a yellowish brown volatile oil, probably identical with the lactone of Sakai (2) which causes contraction of the dog's uterus and intestine, but also produced cardiac depression and severe renal irritation; (b) cane sugar of which the root contains about 40 per cent., and which, together with irritation by volatile material, is probably responsible for most of the diuretic effects produced by the drug, though the crystalline material is weakly diuretic; (c) cardiac depressant material, of undetermined nature.

The crude drug contains volatile oils which act on smooth muscle and produce renal lesions like the emmenagogue oils; the crystalline active principle is water soluble, and therefore this drug may be more effective, though the volatile material makes it little, if any, safer than the emmenagogue group.

193 **(2153)**

The route of migration of schistosoma japonicum in the body of its final host.

By HENRY EDMUND MELENEY and ERNEST CARROLL FAUST.

[*From the Parasitology Laboratory, Department of Pathology, Peking Union Medical College, Peking, China.*]

It is well established that the cercarial form of *Schistosoma japonicum* enters the mammalian host through the skin, and that it passes by way of the blood vessels to the lungs. The route taken by the worms from the lungs to the portal and mesenteric veins where they grow to maturity, has been disputed by various Japanese investigators. Narabayashi[1] concluded that they go mainly by way of the pleural cavity into the mediastinal tissues, and thence through the diaphragm into the abdominal cavity and, by direct penetration, enter the liver and portal vein. Sueyasu[2] claims that they pass from the pleural cavity directly through the diaphragm and into the liver. Miyagawa and Takemoto[3] believe that most of the worms leave the lungs through the pulmonary veins, pass through the aorta and mesenteric arteries into the capillaries of the stomach and intestine and thence into the portal veins. The method used by all these investigators consisted of the examination of serial microscopic sections of mice killed at various intervals after infection.

Owing to the discrepancy of these findings we used somewhat different methods in attacking this problem. A series of ten rabbits was heavily infected, and one was killed on each of the first ten days thereafter. The peritoneal and pleural cavities were washed out, and the blood vessels of the lungs, liver, spleen, gastro-intestinal tract and posterior extremities were irrigated with saline-citrate solution. The popliteal lymph nodes, lungs and liver were cut into fine pieces and washed. The fluids from all these washings and irrigations were then examined for worms.

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1 Narabayashi, H., *Mitteil. Med. Gesell. Kyoto*, 1916, xiii, Nos. 2-3 (Japanese text).

2 Sueyasu, Y., *Kyoto Igaku Zassi*, 1920, xvii, No. 1 (Japanese text with German abstract).

3 Miyagawa, Y., and Takemoto, S., *Jour. Path. and Bact.*, 1921, xxvi, 168.

The young worms were recovered from the liver on the third day. This was as early as they were recovered from the pleural cavity and was one day before they were found in the peritoneal cavity. They were never found in the pleural or peritoneal cavity in large numbers, except in one rabbit, which showed the lesions of chronic passive congestion of the lungs and other viscera. The pleural cavity of this rabbit yielded 171 young worms while in the peritoneal cavity only one worm was found.

In these rabbits petechial hemorrhages were numerous in the lungs from the second day on. They appeared in the mucosa of the stomach and intestine on the third day, and in the muscles, the deeper layers of the skin and most of the other viscera on the fourth day. On the fifth day they were intense in all the viscera᾿ but thereafter became progressively fewer. Serial microscopic sections through a hemorrhage in the gastric mucosa and through the cortex of one kidney of a four-day rabbit, showed young worms in both localities.

A series of four mice was killed on the fourth to the seventh day after infection. Serial microscopic sections of one lobe of the liver of the four-day mouse showed that even this early the worms were all in blood vessels. Most of the blood vessels containing worms could be identified as portal veins. Possibly the remainder were also portal veins, but no bile duct could be seen accompanying them. The worms were distributed evenly throughout the liver. There was no tissue reaction or hemorrhage in the liver such as there was in other organs. The diaphragm of each of these four mice was sectioned serially. The only worms found were one on the fourth day and two on the seventh. The pleural cavity in these mice showed worms on the fourth, fifth and sixth days. The peritoneal cavity showed no worms in any of these mice.

As a result of these experiments we conclude that the principal route of migration of *Schistosoma japonicum* from the lungs to the liver is by way of the pulmonary veins, aorta, mesenteric arteries and mesenteric and portal veins.

194 (2154)

The transmission of tetanus antitoxin through the placenta.

By CARL TEN BROECK and JOHANNES H. BAUER.

[*From the Department of Pathology, Peking Union Medical College, Peking, China.*]

In a previous paper we showed that when tetanus bacilli are present in the stools of man an appreciable amount of antitoxin can be found in the blood. We have used this fact to explain in part the comparatively low incidence of tetanus infections in Peking where approximately a third of the population are carriers of tetanus bacilli. It is of theoretical as well as of practical interest to know whether this antitoxin passes the placenta and we are grateful to Dr. J. P. Maxwell, who has supplied us with the specimens necessary for such a study.

The stools of fourteen of the mothers examined failed to show tetanus bacilli and not one of their sera was able to neutralize two M. L. D. of toxin. Tests on the sera of their children were likewise negative.

Six of the mothers proved to be carriers of tetanus bacilli and the results obtained from the examinations of the maternal and child's (cord) bloods are given in the table.

Hospital number	Child's serum 0.1 c.c.		Mother's serum 0.1 c.c.	
	Neutralizes	Fails to neutralize	Neutralizes	Fails to neutralize
	M. L. D.* toxin	M. L. D.* toxin	M. L. D.* toxin	M. L. D.* toxin
1487	5	10	5	10
1599	25	50	25	50
1668	25	50	25	50
2133	25	50	10	25
2164	25	50	....	5
4009	5	10	5	10

* Field mice were used as test animals.

It will be seen that, with one marked exception, when tetanus antitoxin was found in the mother's serum it was also present in the cord blood and that, in the majority of cases, the level in the two bloods was approximately the same.

Recent experiments have shown that the amount of antitoxin necessary to neutralize 10 M. L. D. of our toxin is slightly less than 0.01 U. S. units. Since the tests for antitoxin in the serum were made with only 0.1 c.c. this means that two of the babies were born with approximately 0.25 units, one with 0.1 units, two with 0.05 units and one with no appreciable antitoxin per c.c. of serum. Although we have been unable to study the colostrum we should expect to find antitoxin there whenever we find it in the serum, thus giving the child an additional supply.

Since the antitoxin level in the mother's and child's bloods is, in the majority of cases, at approximately the same level it seems probably that the placenta is permeable to this antibody. It indicates also that antitoxin has a much simpler structure than the other so-called immune bodies which fail to pass this organ.

195 (2155)

The occurrence of intranuclear inclusion bodies in certain tissues of the rabbit inoculated directly with the virus of herpes labialis.

By ERNEST W. GOODPASTURE and OSCAR TEAGUE.

[*From the William H. Singer Memorial Research Laboratory, Allegheny General Hospital, Pittsburgh, Pa.*]

B. Lipschütz has described in experimental herpetic keratitis of rabbits intranuclear, acidophilic bodies, staining readily with eosin, variable in size and shape· but usually round or oval and separated from the nuclear membrane by a narrow clear space. They may appear homogeneous or faintly granular. He found these bodies constantly in the nuclei of the epithelial cells and considered them pathognomonic of this lesion. Rarely similar bodies were encountered in the nuclei of connective tissue cells of the lesion. He succeeded in demonstrating these bodies also in the conjunctiva of the inoculated rabbit's eye and in herpetic vesicles of human skin.

We have attempted to determine in what tissues of the rabbit the virus of herpes labialis will take as determined by the presence of the above intranuclear bodies at or near the sit of inoc-

ulation. The procedure adopted was as follows: A virus obtained from a herpetic vesicle of the human lip was inoculated upon the scarified cornea of a rabbit and was transferred from cornea to cornea at two- or three-day intervals. The intranuclear bodies were found characteristically in corneas thus inoculated. Purulent secretion was collected twenty-four hours after inoculation of a cornea, suspended in saline solution and injected in small quantities with a hypodermic syringe into rabbits as follows: directly into the testicle, into the brain after trephining the skull, and into the abdominal organs after laparotomy. The tracheal mucosa, the abdominal skin and the muco-cutaneous border of the lip were scarified and purulent conjunctival secretion was rubbed into the scarifications. The inoculated areas were excised twenty-four hours after inoculation, fixed in Zenker's solution and stained with haematoxylin-eosin and with methylene-blue-eosin.

Characteristic intranuclear bodies like those described by Lipschütz have been found in the brain, trachea, testicle, adrenal, liver, muco-cutaneous border of the lip and skin of the abdomen. The bodies were observed in both nerve cells and glial cells of the brain, in the ciliated epithelium of the trachea, in the interstitial cells of Leydig of the testicle, in the cortical cells of the adrenal, in the parenchymatous cells of the liver and the squamous epithelial cells of the skin. Only within cells within the lesion could these bodies be demonstrated. Herpetic virus was shown to be present in the inoculated brain, testicle and adrenal by inoculation of the rabbit's cornea.

We believe that demontstration of these characteristic intranuclear bodies within the nuclei of cells in the inoculated area is diagnostic of a take' indicating proliferation of the virus of herpes labialis locally.

196 (2156)

The antiseptic action of ethoxyquinolin, chitenin and H-acid.

By A. D. HIRSCHFELDER, HERMAN H. JENSEN and W. W. SWANSON.

[*From the Department of Pharmacology, University of Minnesota.**]

In 1911 Morgenroth and his collaborators[1] found that when hydrocuprein, a quinine derivative, was changed to ethyl hydrocuprein the substance acquired specific pneumococcicidal powers, both in vitro and in vivo. However, many clinicians have found that in the treatment of lobar pneumonia ethyl hydrocuprein is too toxic and that changes in the optic nerve and sometimes permanent blindness may result from its use.

Two years ago Pankow and Hirschfelder[2] investigated two parallel series of simple aromatic substances, hydroxy compounds and the corresponding ethoxy compounds, in order to determine whether in these simple compounds also, the introduction of an ethoxy group would yield increased pneumococcicidal action. The experiments showed, however, that this is not the case. In the series reported in this paper we have tested the action of 8-oxyquinolin sulphate
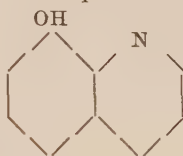
and 8-ethoxyquinolin sulphate
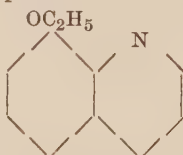
[1] Morgenroth, J., and collaborators, *Berl. Klin.* 1911, lxxi, 501; *Ibid. Ztschr. f. Immunitatsforchung, etc.,* 1912, xv, 610; 1920, xxix, 217.

[2] Hirschfelder, A. D., and Pankow, L. J., Proc. Soc. Exper. Biol. and Med., N. Y., 1922, xix, 64.

A few loopfuls of pneumococcus suspension were added to the solution of the substance in 0.9 per cent. NaCl, and after the desired interval transpired to rabbit blood agar plates and incubated 24 hours. We find that, like the aromatic compounds, there is practically no difference in the action of the two compounds upon the pneumococcus. A ten-minute exposure to 1/5000 solution in 0.9 NaCl kills the cocci. The streptococcus is less sensitive requiring a 1/1000 solution, but no difference is observable between the two compounds.

An attempt was made to produce a quinine derivative having a toxicitiy less than quinine. Chitenin, which differs from quinine in having a —COOH group in place of the vinyl —CH=CH$_2$ group in the side chain was prepared by oxidizing quinine according to the method of Skraup.[3] We have found that this substance is from three to four times less toxic than quinine on subcutaneous injection (minimum lethal dose of Chitenin=2.2^G to 4.2^G per Kg. rat; minimum lethal dose of quinine=0.7^G per Kg. rat).

The antiseptic actions of ethylhydrocuprein, quinine and Chitenin are shown in the following table:

TABLE

+ = growth
— = no growth

Pneumococcus (3 separate tests made)

Ethylhydrocuprein, HCL...... $\dfrac{1}{10,000}$ sol. 5 min. $\dfrac{-}{+}$ 10 min. $\dfrac{-}{-}$ $\dfrac{1}{5000}$ sol. 5 min. $\dfrac{-}{-}$

Quinine, HCL...... $\dfrac{1}{1000}$ sol. 5 min. $\dfrac{-}{+}$ 10 min. $\dfrac{-}{-}$ $\dfrac{1}{500}$ sol. 5 min. $\dfrac{-}{-}$

Chitenin, HCL...... $\dfrac{1}{200}$ sol. 5 min. $\dfrac{+}{+}$ 10 min. $\dfrac{+}{-}$ 1% sol. 5 min. $\dfrac{+}{-}$

$\dfrac{+}{-}$ 10 min.

Chitenin, Na salt...... $\dfrac{1}{500}$ sol. 10 min. $\dfrac{+}{+}$ 30 min. $\dfrac{+}{-}$

H acid...... $\dfrac{1}{500}$ sol. 5 min. $\dfrac{-}{-}$; $\dfrac{1}{1000}$ 30 min. + (24 hrs. —)

Streptococcus

Ethylhydrocuprein, HCL...... $\dfrac{1}{1000}$ 5 min. $\dfrac{--}{++}$ 10 min. $\dfrac{-}{-}$; $\dfrac{1}{2000}$ 5 min. $\dfrac{+}{+}$

10 min. $\dfrac{+}{-}$; $\dfrac{1}{5000}$ 10 min. $\dfrac{++}{+}$

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[3] Skraup, Zd., *Annalen der Chemie*, 1879, excix, 344.

Quinine, HCL................1% 5 min. $\dfrac{-}{+}$ 10 min. $\dfrac{-}{-}$; $\dfrac{1}{1000}$ 10 min. $\dfrac{\pm}{+}$ 30

min. $\dfrac{-}{-}$

Chitenin, HCL................1% 5 min. $\dfrac{+}{-}$ 10 min. $\dfrac{-}{-}$; $\dfrac{1}{1000}$ 5 min. $\dfrac{+}{+}$ 10

min. $\dfrac{?}{+}$ 30 min. $\dfrac{-}{-}$

Chitenin, Na salt................$\dfrac{1}{500}$ 10 min. $\dfrac{+}{+}$ 30 min. $\dfrac{-}{-}$

H acid................$\dfrac{1}{200}$ 30 min. + (24 hrs. —; $\dfrac{1}{1000}$ 24 hrs. +)

Staphylococcus

Quinine, HCL................$\dfrac{1}{200}$ 5 min. +, 10 min. —; $\dfrac{1}{1000}$ 30 min. +

Chitenin, HCL................$\dfrac{1}{50}$ 5 min. +, 10 min.?, 30 min. —

H acid................$\dfrac{1}{200}$ 5 min. —; $\dfrac{1}{500}$ 5 min. +, 10 min. —

Bacillus Coli

Quinine, HCL................1% 5 min. —; $\dfrac{1}{200}$ 5 min. +, 10 min. $\dfrac{-}{?}$; $\dfrac{1}{500}$

Chitenin, HCL................2% 5 min. +; 10 min. +, 30 min. —; $\dfrac{1}{200}$ 30

min. +

Bacillus Typhosus

H acid................$\dfrac{1}{200}$ 30 min. $\dfrac{+}{+}$

Quinine, HCL................$\dfrac{1}{200}$ 5 min. —; $\dfrac{1}{500}$ 5 min. +

Chitenin, HCL................2% 10 min. +, 30 min. —; 1% 30 min. +

H acid................$\dfrac{1}{200}$ 10 min. +, 30 min. —; $\dfrac{1}{500}$ 30 min. +

In these studies it is evident that the Gran staining cocci are more sensitive to the acid chitenin than are the negatively staining bacilli, coli and typhosus. This fact is in striking contrast to the results which Churchman[4] has obtained with the basic and the acid anilin dyes in which the Gram staining organisms are

more sensitive to the basic dyes than to the acid dyes, but the Gram negative bacili are more sensitive to the acid dyes.

In order to study this questions further we have tested the antiseptic action of H acid (aminonaphthol disulphonic acid),
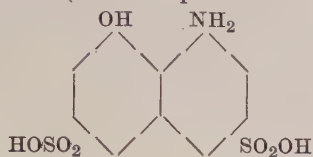
an acid which is present in both trypan blue and the new trypanosome remedy (Baeyer 205[5])* The results are shown in the table above.

It is evident that the Gram positive cocci tested here are more sensitive to H acid than are the Gram negative bacilli, and therefore that Churchman's findings cannot be regarded as evidence of an entirely general rule.

197 (2157)

The synthesis and excretion of hippuric acid: The glycine factor.

By F. B. KINGSBURY.

[*From the Biochemical Laboratory of the Department of Physiology, University of Minnesota.*]

The rate of synthesis and excretion of hippuric acid in normal individuals and those with various renal disorder has already been studied by Kingsbury and Swanson[1] and this study made the basis of renal function test. In this work 2.4 grams of sodium benzoate was the dosage regardless of the size or weight of the individual. In the present paper the original work has

4 Churchman, J., *Jour. Exper. Med.*, 1913, xvii, 373; PROC. SOC. EXPER. BIOL. AND MED., 1922, xix, 288-31.

* A sample of pure H acid was kindly prepared for us by Drs. Derick and Strachan, of the laboratories of the National Aniline Company, and the bacterial cultures were furnished by Dr. J. F. Noble, to whom we extend our sincere thanks.

5 Bayer 205, *Science*, 1922, lvi, 514.

1 Kingsbury, F. B., and Swanson, W. W., *J. Biol. Chem.*, 1921, xlv, 4; *Arch. Int. Med.*, 1921, xxviii, 220.

been extended using dosages of sodium benzoate based on body weight in one series of 21 (50 milligrams per kilo) and on body surface in another series of 66 (1.8 grams per square meter of body surface). Body surfaces were obtained from the height-weight formula and chart of Dubois and Dubois.[2] The dosage based on surface was obtained by calculation after determining the body surface of one individual of average size and weight who had had 50 milligrams of benzoate per kilo of body weight. All individuals of both series were medical students who made the tests on themselves as part of their work in Physiolical Chemistry and whose urine specimens I made check analyses on by the method of Kingsbury and Swanson.[3] The benzoate was ingested quantitatively the first thing in the morning, having voided the night urine immediately before. Breakfast was omitted. The total water intake was 500 c.c. in every case. The urine was collected for the next three hours and immediately analyzed. The small and variable 3 hour normal output of hippuric acid independent of the ingested benzoate is included in these figures. Experience has shown that it is unnecessary to determine this and correct for it in the practical application of the test. In the first series of 21 it was noted that the groups of heavier individuals generally put out a smaller amount of hippuric acid per kilo of body weight than did the groups of lighter individuals, although all received the same dosage of sodium benzoate per kilo of body weight. This is shown in Table 1 in the form of percentages of excretion for the different weight groups.

This indicates that the actual amount of functioning tissue in the kidneys is less in its relation to body weight in the heavy person than in the light. This was also shown by a fairly high, but negative correlation coefficient 0.50, between the per cent. excretion and the body weight with a probably error of ±22 per cent.

In a second group of 66 normal individuals given benzoate, 1.8 grams per square meter of body surface, it was noted that the average percentage of excretion for groups of different body weights was nearly constant, indicating that the actual amount of functioning kidney substance is more nearly in proportion to the body surface than to the body weight. Calculations have shown that by using the square root of the body weight as a

2 Dubois and Dubois, *Arch. Int. Med.*, 1917, xvii.

3 Kingsbury, F. B., and Swanson, W. W., *J. Biol. Chem.*, 1921, xlviii, 13.

basis for the benzoate dosage that nearly the same amount is obtained for any one individual as by the body surface plan. This agrees with the results obtained by Austin, Stillman and Van Slyke,[4] who found that the square root of the body weight was more adequate than the body weight itself in their constant for urea excretion. The correlation coefficient between the milligrams of hippuric acid excreted per square meter of body surface and the volume of urine excreted on the same basis was small, 0.13, negative and with a very high probable error, ±64 per cent., indicating that in general there it little relationship between the excretion of hippuric acid and of water and that there is a slight tendency for the kidney that shows the best output of hippuric acid also to show the best concentrating ability. In the first group of 21 quite the reverse of this was true' namely that the greater volume of water that was excreted in a test was accompanied by a greater relative amount of hippuric acid. This was well shown by correlating the milligrams of hippuric acid excreted per kilo of body weight with the volume of urine excreted on the same basis. The coefficient obtained was 0.42, positive, and with a probable error of ±28 per cent. A comparison of some of the findings with the two groups are shown in Table 1.

It will be noted from Table 1 that 91 per cent. of the individuals of the group of 66 were able to excrete more than 85 percent. of hippuric acid theoretically obtainable from the ingested benzoate. Values lower than 80 per cent. in this test for renal function should be regarded as indicating probable renal insufficiency.

Two normal individuals in a total of 89, given benzoate according to the body surface plan gave evidence that glycine was not furnished with sufficient rapidity for the synthesis and excretion of hippuric acid at the normal rate. The first, a healthy medical student, gave on two occasions tests of 53 and 67 per cent. He was thoroughly examined by Dr. George E. Fahr of the Department of Medicine, who also determined his Van Slyke[4] urea excretion constant. This was within the normal limits. The second student showed benzoate test figures of 69 on two occasions and while not examined physically was apparently in perfect health. Glycine in amount equivalent to the benzoate was

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4 Austin, J. H., Stillman, E., and Van Slyke, D. D., *J. Biol. Chem.*, 1921, xlvii, 91.

given with the benzoate to each of these students in subsequent tests. The first showed 90 per cent. and the second 84 per cent. It should be noted that there was an interval of at least a week between any two of these tests on the same individual. The benzoate dosage in each case was approximately 50 milligrams per kilo of body. Lewis and Griffith[5] gave rabbits the enormous dosage of 1 gram of sodium benzoate per kilo of body weight and found that the simultaneous administration of glycine markedly increased the rate of synthesis and elimination of hippuric acid. This was to have been expected since the dosage which they used was approaching the lethal dose of 1.7 grams per kilo. In previous tests using 2.4 grams of benzoate I had tried the effect of the simultaneous administration of glycine without result in the case of normal human beings, and I was therefore surprised that with the somewhat larger dose of 50 milligrams per kilo, but still a relative small dose, to find two persons out of a series of 89 who showed this peculiar condition. This apparent difficulty in certain persons of readily supplying glycine must be taken into account in making benzoate tests: Experiment with normal given benzoate per body surface basis shows administration of glycine increases rate of hippuric acid output in two hours' interval markedly but slightly in three hours interval. It will be necessary to supply sufficient glycine in all tests to rule out the possibility of an occasionally low benzoate test being due to this glycine factor. Gelatine can probably be adequately substituted for glycine for this purpose if given in proper amount and a short time before the administration of the benzoate to allow time for digestion.

TABLE I.

Body wt. kilos.	Group 1, 21 cases		Group 2, 266 cases		Group 2		
	No. of cases	Aver. H. A. % excreted	No. of cases	Aver. H. A. % excreted	% excretion	No. of cases	% of total
41- 50	1	100	1	96	80- 85	6	9
51- 60	5	91	16	91	86- 90	20	30
61- 70	11	90	26	96	91- 95	22	33
71- 80	3	83	21	91	96-100	18	28
81- 90	1	85	1	92			
91-100	0		1	100			

ABSTRACTS OF COMMUNICATIONS.

Western New York Branch.

Sixth meeting.

Rochester, New York, April 14, 1923.

198 (2158)

A method for the study of liver metabolism.

By KARL F. CORI and G. T. CORI (by invitation).

[*From the State Institute for the Study of Malignant Disease, Buffalo, N. Y.*]

A method has been described which allows one to obtain 8-10 pieces of liver of the size of one gram under entirely physiological conditions. A special apparatus has been constructed consisting of one part which is sewn in into an opening of the peritoneal cavity just below the sternum and of a cover which can be screwed off. Three days after the operation the animal can be considered as normal. It has a normal food intake. No disturbances in the motility of the bowels have been observed. By screwing off the cover of the window nearly every lobe of the liver can be reached. Bleeding is entirely avoided by using slightly heated scissors or Squibbs thromboplastin. The animal shows no signs of excitement or pain. No increased adrenaline production with following hyperglycemia occurs. If all operations are performed aseptically one can remove the window and the animal will survive. A further advantage of this method is that solutions can be injected directly into the stomach.

Five lantern slides were shown, illustrating the usefulness of this method. In two experiments the free sugar in the liver was determined simultaneously with the bloodsugar after the administration of adrenaline. One experiment was given showing the effect of ingestion of 5 grams of glucose on the free sugar and glycogen in the liver and the bloodsugar. Two similar experiments were shown when glucose and iletin were given at the same time.

199 **(2159)**

Friedlander bacillus bacteremia.

By O. W. H. MITCHELL.

[From the Department of Bacteriology and Hygiene, Syracuse University, Syracuse, N. Y.]

Rare instances of Friedlander bacillus bacteremia have been reported.   Recently such an infection was encountered.   The following are the most important data.   An Italian girl, eight years old, was taken ill during the evening of March 19.   All day she had been apparently well and ate a hearty supper with a large portion of egg-plant.   At bedtime it was noticed that she was somewhat shivery and feverish but did not appear definitely ill.   Went to sleep but at midnight was nauseated, vomiting and shivering.   Her people were up with her during the night and in the morning she appeared quite ill.   She was sleepy and dull.   There was considerable trembling and some stiffness of the neck.   She mumbled a great deal.   A physician suspected meningitis and sent her to the University Hospital of the Good Shepherd.   An examination of the spinal fluid gave normal findings.   The patient was under the care of Drs. Cornell Smith and A. C. Silverman of the Pediatric Service.   The physical examination was negative except for the following: "Examination of the lungs shows some harsh breathing in the upper right lobe, posteriorly.   Occasionally coarse, moist rales are heard.   Respirations are rapid and shallow, 52 to the minute.   Pulse 160, temperature 103."

A blood culture was made on March 21 at 4 p. m.   The following morning growth was observed.   The flask had the odor of colon bacillus growth.   In the hanging drop there was apparently no true motility.   Subcultures were made on the usual media including lactose, dextrose and saccharose broth.   The subcultures on the solid media gave the appearance of the colon bacillus.   The growth was not particularly viscid.   It was not sticky or tenacious.   Subcultures were more typical and grew more luxuriantly.   After standing five days the growth was tenacious.   All of the sugars were fermented rapidly and the closed arm was almost completely filled with gas.   This strong-

ly suggested the Friedlander group. Capsule stain from the subculture was negative but intraperitoneal inoculation in the white mouse yielded typical capsulated bacilli. The mouse was very ill when killed at the end of 18 hours. Gelatin was not fluidified. The organism was definitely a member of the Friedlander group. As the only physical finding suggested the possibility of a lung lesion the isolation of the bacillus pointed to that region as the most likely diseased area. The X-ray examination on March 26 showed marked density of the right upper chest from the second to the sixth rib. On March 30 this density had decreased to a mere cloudy appearance.

After a most stormy career for six days during which time the temperature reached 106.8°F., there was a rather sudden change for the better and the patient made a rapid recovery.

200 (2160)

Thyroparathyroidectomy in the rabbit.

By SUTHERLAND SIMPSON.

[*From the Department of Physiology and Biochemistry, Cornell University Medical College, Ithaca, N. Y.*]

In reviewing the literature on the parathyroids, one is struck by the lack of agreement in the results obtained by different experimenters following complete removal of the glands, in animals of the same species. For example, Gley, in 1892, when he rediscovered the parathyroids, in his first series of experiments on rabbits, removed the thyroid and parathyroids from sixteen animals. In fourteen of these acute symptoms developed very rapidly and death followed within a day or two. In the same year Moussu repeated Gley's experiment on rabbits and of the eleven individuals on which he performed the complete operation not one showed the acute tetany described by Gley.

In late August and early September 1920, the writer thyroparathyroidectomized seventeen half-grown rabbits, keeping six of the same litters as controls. Of the seventeen, one died within

twenty-four hours in acute tetany, another succumbed in two days and the remaining fifteen lived for months. Some were killed for want of space, and several that were allowed to live showed the chronic changes which follow thyroidectomy.

In January, 1921, the same operation—complete thyroparathyroidectomy—was performed on twenty-four rabbits, most of them adults. Five died in tetany one day after the operation—tracings of the muscular contractions were obtained from two,—four died on the second day, six had succumbed between the second and the tenth days, one on the eighteenth day, and the remaining eight lived until they were killed months afterwards. In this second series the proportion showing acute symptoms was much greater than in the first.

Accessory parathyroid tissue is said to be present in the rabbit fairly frequently. This is small in amount, no doubt, but the first explanation one thinks of to account for the survivals is that some of this tissue has been inadvertently left behind. In the light of work recently reported by Dragstedt and by Luckhardt, however, another explanation is suggested. These observers found that in the dog the symptoms could be controlled by the diet. The toxic substances leading to tetany, they believe, are produced chiefly in the gastro-intestinal tract, arising through the activity of the proteolytic group of intestinal bacteria, and are probably, for the most part protein split products of the nature of amines. A diet rich in proteins is contraindicated therefore, and putrefactive changes in the intestine are to be prevented.

The diet of the first group of rabbits consisted almost entirely of green clover, while in the case of the second group it was mainly oats and cracked corn, although small quantities of cabbage were included. This dry feed would probably be richer in protein and would also tend to induce constipation and so putrefactive changes. This may conceivably account for the high proportion of acute (parathyroid) cases in the second or winter-fed group.

201 (2161)

Further observations on the chemical and physical properties of insulin.

By H. A. PIPER, H. A. MATTILL and JOHN R. MURLIN.

[*From the Physiological Laboratory of the University of Rochester, Rochester, N. Y.*]

Probably all active workers with insulin have observed considerable variation in the potency of the final product by whatever method obtained. Experience in this laboratory had led us to reinvestigate especially the influence of the exact H ion concentration upon potency at the several stages of the aqueous method.[1]

It has been found that after thorough extraction in N/5 HCl neutralizing with N/1 NaOH to P_H of 5.0 to 5.7 gives the largest yield of potency in the first filtrate.

The reaction is controlled both titrometrically to phenolphthalein and electrometrically (error $\pm 0.2 P_H$). Stopping at a P_H of 4.0 to 4.4 gives more rapid filtration but not so much available potency at once. However if the reaction after filtration is at once readjusted to 5.3 or thereabouts the potency is rendered available or, if it be allowed to stand for 24 hours at P_H 4.0 to 4.4 the potency increases. In fact the filtrate may be kept at room temperature until there is an abundant growth of bacteria and yeasts without destroying the potency.

In the final concentration it has been found that potency is best preserved in a fairly acid medium .06 to .08 N HCl; though a reaction as high as P_H 5.7 preserves for several weeks if an antiseptic like tricresol is added at once.

Pasteurization temperature maintained for half an hour does not destroy the potency. In fact the concentrated extract purified of proteins can be heated to 80° for half an hour, the exact effect depending upon the reaction. At a P_H of 6.2 to 7.0 or higher heating to this point seems regularly to increase the potency on rabbits. Heating at a P_H of 4.4 to 5.7 the potency is usually diminished or carried down with the coagulum if proteins are

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[1] Murlin, Clough, Gibbs and Stokes: *Jour. Biol. Chem.*, 1923.

present.   Heating the first filtrate (containing much protein) at P_H of 4.0 does not always produce a coagulum and if not, may increase the potency.

202 (2162)

Precipitation reactions of insulin.

By C. P. KIMBALL, R. S. ALLEN and H. A. PIPER.

[*From the Physiological Laboratory, University of Rochester, Rochester, N. Y.*]

We here report the results obtained by the addition of various reagents to the aqueous solution of insulin as it is prepared for injection in human cases.   Potency tests were made on normal rabbits, the dose given being equivalent to 20 gms. of pancreas except in a few of the earlier experiments where the equivalent of 40 gms. was taken.   Only those instances where some positive test was obtained with the same material treated at the same time are reported.

Up to date 29 reagents embracing a wide range in chemical nature, have been tried of which two (formaldehyde and ethyl acetate) gave no precipitate of any kind.   Next comes a list of 6 which gave a definitely negative result, *i. e.,* contemporary experiments with the same material gave some positive tests with other reagents.   It should here be explained that on the addition of the reagents the precipitate is thrown down by centrifuge· taken up in sterile water and injected, the supernatant liquid is dried down, with or without dialysis, depending on the nature of the precipitant, and similarly dissolved and injected.   The following 6 gave definite negatives: ether, petroleum ether, toluene, xylene, chloroform and cadmium chloride.   Five more are probably destructive to the principle, phosphotungstic, phosphomolybdic and tannic acids, $MgSO_4$ and $NaSO_4$ although the evidence is not yet conclusive.   Mechanical difficulties were encountered with phenyl hydrazine, pyrogallic and picric acids, which have eliminated them from our list.   Too little has been done with UO_2Ac_2, $Zn\ SO_4$ and $NaCl$ to justify any inferences.

Turning now to the positive results we find that absolute ethyl alcohol is unique in being the only reagent encountered so far

that throws down a precipitate from aqueous solution but leaves the insulin in solution. Eight of the nine remaining reagents have given consistent and good positive precipitations. Of these ammonium sulphate is the only inorganic reagent and our results with it have been far from startling, although Shaffer claims that by means of one-half saturation with it insulin is thrown out of solution in the globulin fraction. Acetone, trichloracetic acid and the six alcohols, methyl, isopropyl, n-propyl n-butyl, n-amyl, and n-caprylic have rarely failed to yield a potent precipitate.

In the table all potency values except the first few represent the drop in the blood sugar of the normal rabbit brought about in a period of two hours by the injection of the equivalent of 2 c.c. of the original or 20 gms. pancreas.

TABLE I.

EFFECT OF PRECIPITATION ON POTENCY*

Extract No.	Precipitant 5 vols. added	Original Potency	Potency of precipitate	Potency of filtrate
84-2	Absolute alcohol	—.045	+.017	—.085
84-2	Acetone	—.045	—.034	±.000
84-2	Toluene	—.045	—.003	—.029
84-2	Chloroform	—.045	—.013	—.004
75-3	Absolute alcohol	—.061	—.010	—.070
75-3	Ether	—.061	+.011	—.018
75-3	Chloroform	—.061	—.015	—.001
87-3	Acetone	—.047	—.036	—.009
87-3	Acetone reppt.	—.047	—.049	
101AB	n-Butyl alcohol	—.072	—.075	—.081
101AB	n-Amyl alcohol	—.072	—.082	+.003
105	Trichlor-acetic	—.087	—.069	+.003
106B	n-Butyl alcohol	—.028	—.039	—.003
106B	n-Amyl alcohol	—.028	—.052	—.009
106B	n-Caprylic alcohol	—.028	—.050	
114A	Trichloracetic	—.007	—.046	
114A	Ammonium sulphate[1]	—.007	—.030	
120I	n Butyl alcohol	—.056	—.057	
120I	n-Propyl alcohol	—.056	—.071	
120I	iso-Propyl alcohol	—.056	—.041	
120I	Methyl alcohol	—.056	—.056	—.004
120I	Methyl alcohol	—.056	—.033	+.003
119 III	Ammonium sulphate[1]	—.037	—.024	
119 III	Ammonium sulphate[2]	—.037	—.013	
119 III	Magnesium sulphate[1]	—.037	+.015	
119 III	Magnesium sulphate[2]	—.037	no ppt.	
119 III	Sodium chloride[1]	—.037	+.012	
119 III	Sodium chloride[2]	—.037	—.051	
119 III	Sodium sulphate[1]	—.037	.000	
119 III	Sodium sulphate[2]	—.037	+.010	

[1] one-half saturation   [2] complete saturation

* These results are chosen at random. They are not to be taken as bases for the conclusions, a complete report at the present time requiring more space than its value would warrant.

There are a few instances of considerable rise, far beyond any experimental error. Although these occur only in those cases where inorganic salts were present and can probably be explained on the grounds that these were not entirely removed, the facts are not without interest. Using $CdCl_2$ in an attempt to remove lecithin, the filtrate of No. 93.2 showed a rise of 339 mg. against an original drop of 96 mg. The precipitate thrown down by $(NH_4)_2SO_4$ in No. 108x showed a rise of 79 mg. against an original drop of 69.

There are other instances where a slight rise was consistently found, but being within the limits of experimental error the results may not be significant. This is noticed especially in the case of the precipitate thrown down by alcohol and in the filtrates, when inorganic reagents are used even though dialyzed and though the evaporated solution shows no microscopic evidence of the crystals of the substance used.

A series of experiments was conducted with acetone as a precipitant with three equivalent portions of varying acidity, one made slightly acid, the second practically neutral and the third slightly alkaline. In those results which gave positive tests there was a very marked gradation first in the potency and second in the nitrogen content. The most acid of the three yields the most potent precipitate and has the lowest nitrogen value.

TABLE II.

EFFECT OF REACTION ON PRECIPITATION BY ACETONE

Extract Number	Precipitant	Reaction or Pн	Original Potency	Potency of ppt.	Nitrogen %
98-2	Acetone	slightly acid	—.090	—.039	—
98-2	"	neutral	—.090	—.039	—
98-2	"	slightly alkaline	—.090	—.023	6.54
99-2A	"	5.8	—.063	{ —.094 } —.071	4.26
99-2A	"	6.7	—.063	{ —.030 } —.023	4.92
99-2A	"	8.8	—.063	{ —.041 } —.053	6.08
103-1	"	5.7	—.046	{ +.007 } —.008	10.3
103-1	"	6.7	—.046	{ +.004 } —.003	10.2
103-1	"	7.8	—.046	{ +.015 } —.028	10.0

203 (2163)

Relative amounts of insulin obtained by extraction and by perfusion of the pancreas.

By H. D. CLOUGH and J. R. MURLIN.

[*From the Physiological Laboratory of the University of Rochester, Rochester, N. Y.*]

We have employed two essentially different methods for obtaining insulin from the excised pancreas of various animals: First, extraction with different media after maceration of the pancreases; and secondly, perfusion of the intact organs with various solutions. In a series of twenty-nine perfusions done during the past ten months we have purposely varied the many factors involved—such as the composition of the perfusion fluid, the temperature of the chamber containing the organs, the rate of perfusion, volume of perfusate, time of perfusion, and perfusion pressures—within wide limits· in order to select the simplest method which is efficient. A comparative analysis of these various factors leads us to conclude that the simplest efficient method is that of continuous gravity perfusion with 0.2 per cent. HCl at or somewhat above body temperature (37C. to 45C.), under a pressure of 120 mm. Hg, for a period of one hour.

In determining the potency of preparations we have used a dose of two cubic centimeters of final concentrated product administered subcutaneously to normal rabbits. Blood is taken from the ear veins before the injection and again two hours after the injection. A drop of 70 milligrams in blood sugar is taken as a rabbit unit and on this basis the yield in rabbit units per kilo of pancreas is calculated.

The perfusion method appears to give about three times as much insulin (estimated as Rabbit Units) per kilo as is obtained by the extraction methods employed and gives a product which is much more easily and quickly concentrated because of the absence of the large amounts of protein and extraneous material which were obtained when extraction processes were used.

RELATIVE AMOUNTS OF INSULIN OBTAINED BY EXTRACTION AND BY PERFUSION

Number	Method of preparation	Fluid	Type	2 hr. Blood Sugar Drop (mg) in Normal Rabbit	R. U.* per kilo of Pancreas	Gms. of Pancreas per R. U.
89 (2)	Extraction	N/5 HCl—23 days (after maceration)	Ox	0.089	76	13.0
93 (2)	Extraction	N/5 HCl—18 hrs. (continuous grinding)	Ox	0.067	96	10.4
121-I (x)	Extraction	N/5 HCl 18 hrs. (continuous grinding)	Ox	0.071	100	10.0
38	Perfusion	0.1% HCl (1 hr.—gravity)	Pig	0.092	262	3.8
34B	Perfusion	Ringer's Sol. + 0.2% HCl + 5% ether (2 hours)	Pig	0.100	286	3.4
26	Perfusion	Ether—5% HCl 0.2% (2 hours)	Pig	0.104	300	3.3

*Rabbit Unit = 70 mg. blood sugar drop in two hours in a rabbit weighing two kilos.

204 (2164)

Clinical observations on the use of the anti-diabetic substance.

By C. B. F. GIBBS and C. C. SUTTER.

[From the Physiological Laboratory of the University of Rochester, Rochester, N. Y.]

Following the attempts to improve the condition of diabetic patients by administering pancreatic extracts by mouth and by duodenal tube, purified preparations have been given hypodermatically and intravenously. Most of these preparations were made by acid aqueous extraction; the fresh pancreas of ox or pig being ground in a mill with acid media and the resultant filtrate concentrated and purified. Other material was obtained by perfusing the pancreas with acid aqueous media and then concentrating and purifying the perfused fluid.

The patients on whom the preparations were used were mostly of severe type not being previously controlled by dietetic regulation. All of these cases were hospitalized in order that rigid control of diet, urinary excretion and blood sugar levels could be obtained. The cases in this series were 12 adults ranging from 26 to 55 years of age.

Charts of the clinical data on these cases and graphs showing the changes in blood sugar levels and urinary sugar excretion were demonstrated.

CONCLUSIONS

1. Potent preparations of the internal secretion of the pancreas can be made by aqueous acid extraction and perfusion.

2. These preparations were made non-toxic and non-irritant for hypodermatic and intravenous administration.

3. The hyperglycemia can be markedly reduced and the glycosuria and acetonuria can be made to disappear by means of these preparations.

4. The clinical condition of patients can be markedly improved, *e. g.,* the thirst and polyuria diminished, edema removed, weight gained and mental sluggishness caused to disappear.

5. The carbohydrate tolerance is increased commensurate with the amounts of extracts employed.

6. Dessicated pancreatic extracts taken in capsule form by stomach have thus far not proved to be effective in reducing blood or urinary sugar.

7. Patients in complete coma can be brought back to a rational condition, their acidosis controlled and the non-protein nitrogen and creatinine lowered to a normal level.

8.   Thus far no definite evidence has been obtained to show that the carbohydrate tolerance of a diabetic patient is permanently raised by temporary extract treament.

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205 (2165)

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The degeneration of the testis of rats on a milk diet.

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By H. A. MATTILL and J. S. CARMAN.

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[From the Physiological Laboratory of the University of Rochester, Rochester, N. Y.]

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The degeneration of the testis of rats on rations in which all the protein and vitamines are supplied by milk[1] has also been observed when such rations are supplemented by nucleoprotein and by those proteins of first quality[2] found in kidney and liver. When 2 per cent. or 5 per cent. of these dried tissues' or 2 per cent dried thymus (Parke, Davis) or 2 per cent. yeast nucleic acid was supplied in rations containing 50 per cent. dry whole milk, 15 per cent., lard, 2 per cent., salts, and starch, evidences of degeneration and atrophy appeared as early as 155 days of age. Among 27 animals on such rations, the oldest animal still possessing normal gonads was 139 days of age.   Of 25 animals over 155 days of age only one had gonads whose weight was normal and in this animals there were other evidences of degeneration.   Without exception litter mates of these animals on stock rat food had normal organs.   Aside from the decreased weight which was occasionally even less than one-half the normal for the weight of the animal the degenerating glands have a semitransparent glassy appearance; when ruptured they collapse and there exudes a clear colorless liquid which coagulates like lymph.   Histologically these glands show a profound degeneration of the germinal epithelium and an abundant proliferation of the interstitial tissue together with numerous clear amorphous areas.   In the main these are the features which Allen[3] described as resulting from the absence of water-soluble B in the diet.   Since in our milk rations there can be little or no question as to the adequacy of the vitamin B supply, especially when liver or kidney is added, it would appear that the lack of some other substance than vitamin B, and as yet unrecognized, may be solely or jointly concerned in the disappearance of the reproductive function.

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[1] Mattill, H. A., and Stone, N. C., *Jour. Biol. Chem.*, 1923, lv, 443.

[2] McCollum, E. V., Simmonds, N., and Parsons, H. T., *J. Biol. Chem.*, 1921, xlvii, 235.

[3] Allen, E., *Anat. Rec.*, 1919, xvi, 93.

SCIENTIFIC PROCEEDINGS

206 (2166)

On the presence of a fat-soluble substance in purified casein.

By CASIMIR FUNK.

[*From the Research Laboratory of H. A. Metz, New York City.*]

A sample of casein, purified by three reprecipitations and extraction with alcohol and ether in the cold, yields on extraction with boiling alcohol and cooling a fat-like substance, which attracted our attention. The crystals obtained proved to be free from nitrogen, melted at 52° and gave no cholesterol reactions. On treatment with boiling alcoholic potash the substance was incompletely saponifiable. Various samples of well purified casein gave 0.5-1 per cent. of this material, whereas A-free casein Harris failed us so far in this respect.

The question whether the presence of this fat-like product fed along with the casein, plays a rôle in nutrition experiments, is being taken up in a series of experiments. It follows from the above data, that casein cannot be completely freed from lipins by extraction in cold with alcohol and ether and that the method of purification adopted by the present writer with Macallum (repeated boiling out with alcohol) is probably the most suitable one to use in preparing casein for feeding experiments.

207 **(2167)**

The presence of a blood-sugar reducing substance in yeast.

By CASIMIR FUNK and H. B. CORBITT.

[*From the Research Laboratory of H. A. Metz, New York City.*]

In 1914 one of the present authors reported in collaboration with v. Schoenborn[1] that pigeons kept on a vitamine-free, artificially compounded diet, show complete disappearance of the glycogen in the liver and increased blood sugar. In one particular series of experiments the blood sugar amounted to 0.29 per cent. and no glycogen was found in the liver. In a few pigeons kept on the same diet one dose of vitamine B from yeast was injected intramuscularly with the result that liver was found to contain 0.6 per cent. of glycogen and the blood 0.19 per cent. sugar. This vitamine preparation, which proved to be very potent, was prepared in the following way: an evaporated alcoholic extract of yeast was precipitated with phosphotungstic acid, and the resulting dried precipitate treated with acetone. The insoluble fraction was decomposed with lead acetate and the filtrate freed from adenine by means of picric acid.

This observation gains much interest in view of the recent communications of Winter and Smith and also of Collip on the presence of an insulin-like substance in yeast and other starting materials.

For some time past we have been working on the same problem again. We have found that crude extracts of yeast and rice-polishings possess a blood-sugar increasing rather than decreasing action. When we took, however, yeast grown in the laboratory on a medium rich in vitamine D, then centrifuged and washed the cells, and after heating to 100° injected them subcutaneously into rabbits, we obtained in a number of instances in 3-4 hours blood-sugar decreases which amounted to 30-40 per cent. of the initial value. We agree with Collip that compared with insulin the action of the yeast substance is slow, but on the other hand it lasts longer which might prove of therapeutic ad-

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1 *J. Physiol.*, 1914, cccxxviii, 48.

vantage. We have not as yet succeeded in obtaining similar results with Fleischmann's yeast treated in the same way.

The slow and lasting action of the yeast substance suggests strongly its non-identity with insulin. It seems possible that the substance undergoes slowly a change into insulin. The demonstration of a blood-sugar reducing substance in various foods will perhaps explain the marked hyperglucemia that one of the authors has described in pigeons on a diet devoid of vitamine B (blood sugar of 30 normal pigeons was found to be 0.170 per cent., of 32 animals fed on polished rice 0,243 per cent., with occasional figures as high as 0.3-0.5 per cent.). It may be that the increase of sugar found is due to deficiency of an insulin-precursor in the food. If this should prove to be true diabetes might be caused by non-functioning of the pancreas, or theoretically, at least, by a deficiency of the insulin precursor in the food.

208 (2168)

Extraction of vitamines from yeast and rice polishings using various water-miscible solvents.

By CASIMIR FUNK, BENJAMIN HARROW and JULIA B. PATON.

[*From the Biochemical Laboratory of Columbia University, College of Physicians and Surgeons, and the Research Laboratory of H. A. Metz, New York City*]

The comparative values of the following solvents in extracting vitamine from yeast were studied: ethyl alcohol (50, 60, 70, and 80 per cent.), methyl alcohol (60 to 70 per cent.), propyl alcohol (70 per cent.), butyl alcohol (70 per cent.), isobutyl alcohol (70 per cent.), acetone (70 per cent.), methyl ethyl ketone (70 per cent.) and acetic acid (70 per cent.). The extracts and *residues* were tested on pigeons and rats, and the extracts were also tested for their content of vitamine *D* (yeast growth), co-ferment, total nitrogen and total solids.

If inactivity of residue be taken as a criterion, then 70 per cent.

alcohol has proved to be the best solvent for vitamine among the solvents used. If, however, we stress not only the activity of the extract, but also the quantiy of nitrogenous and other impurities accompanying the extract (selecting the one that gives a minimum of such impurities), then the best among the solvents is acetone.

Vitamines *B* and *D* tend to run parallel with one another, so that, as a rule, the higher the content of vitamine *B*, the higher in vitamine *D* is the extract apt to be.

The co-ferment shows no definite relation to either vitamine *B* or *D*.

On the whole, the higher the nitrogen content of the extract, the greater the percentage of total solids, and the greater the activity of the extract.

Using these solvents on rice polishings instead of on yeast, we find that 60 per cent. alcohol is better than 70 per cent.

The extracts from rice polishings seem to be particularly active when tested on rats, and, by comparison, far less so when tested on pigeons.

209 (2169)

Clinical results obtained with bacillus acidophilus.

By NICHOLAS KOPELOFF.

[*From the Department of Bacteriology, N. Y. State Psychiatric Institute, Ward's Island, New York City.*]

1. A series of 30 constipated subjects were under observation before, during, and after treatment with *B. acidophilus*. A comparison of these periods shows that the number of normal defecations has been significantly increased during treatment. The usual daily dose was 1,000 c.c. containing 200,000,000 viable *B. acidophilus* per c.c.

2. The beneficial influence of *B. acidophilus* usually persists for a considerable period of time after treatment has been stopped. Patients have been observed for from one week to about

one year after treatment and almost without exception all have had more normal defecations after, than before, treatment.

3. The use of lactose during and after ingestion of *B. acidophilus* does much to enhance the beneficial effects.

4. A transformation of the intestinal flora from a proteolytic to an aciduric type as shown by microscopic and plate counts may generally be induced. Such transformation is usually accompanied by almost daily defecations regardless of the severity of the constipation.

5. Two cases of diarrhea have been successfully treated by the ingestion of *B. acidophilus*.

210 (2170)

Studies on the nature of bacillus acidophilus therapy.

By NICHOLAS KOPELOFF and PHILIP BEERMAN.

[*From the Department of Bacteriology, N. Y. State Psychiatric Institute, Ward's Island, New York City.*]

1. In order to study the influence of physical and chemical factors, *B. acidophilus* milk was centrifuged and run through a Mandler diatomaceous filter. Thus the chemical constituents were little altered. When fed to constipated patients it was practically without effect. Regular *B. acidophilus* milk ingested subsequently resulted in an increase in the number of normal defecations.

2. *B. acidophilus* milk was sterilized and lactic acid added, thus again approximating the original chemical composition. When fed to constipated patients, little change was noted. Regular *B. acidophilus* milk ingested subsequently resulted in an increase in the number of normal defecations.

3. These data indicate that *B. acidophilus* therapy is essentially bacteriological rather than physical or chemical in nature.

211 (2171)

Effect of certain electrolytes on the buffering power of bacterium coli.[1]

By I. S. FALK and H. J. SHAUGHNESSY.

[*From the Department of Public Health, Yale School of Medicine, New Haven, Conn.*]

In the present studies we have developed a technique for the measurement of the power of the bacterial cell to combine with hydrogen and hydroxyl ions. The bacteria show a general tendency to simulate amphoteric colloids and we have therefore measured their amphoteric property by the use of titration curves. A bacterial suspension in water or in a particular salt solution is titrated electrometrically with hydrochloric acid and with sodium hydroxide and similar titration curves are obtained for the menstruum alone. From these two sets of curves it is possible to calculate a series of "buffer ratios" by taking the quotients of the amounts of acid or alkali added to shift the P_H one unit in the bacterial suspension and in the menstruum alone. Such buffer ratios have been calculated for each P_H unit zone between P_H 2 and P_H 12. The titration cells which have been developed for these experiments are illustrated in the accompany figure. The buffer ratios for *Bact. coli* in distilled water, 0.725, M NaCl, 0.145 M CaCl$_2$ and in 0.580 M NaCl + 0.145 M CaCl$_2$ solutions are presented in Table I.

TABLE I.

Average Buffer Ratios for Bacterium coli in Water and in Salt Solutions.

P_H Zone.	Ratio for *Bact. coli* in:			
	Water.	0.725 M NaCl	0.145 M CaCl$_2$	0.580 M NaCl + 0.145 M CaCl$_2$
3-2	0.85	1.5	1.0	1.2
4-3	0.77	1.0	1.0	1.3
5-4	1.7	1.8	0.75	1.4
6-5	-------	-------	1.0	1.0
7-6	8.2	1.9	-------	1.3
7-8	4.7	1.4	-------	-------
8-9	1.7	0.75	1.2	1.3
9-10	2.4	0.88	1.5	0.75
10-11	0.59	0.92	0.92	0.91
11-12	0.93	0.68	0.91	1.0
Number of experiments	7	3	3	4

Our findings may be summarized in the following categorical manner:

1.   In distilled water suspensions of *Bacterium coli* show a marked tendency to resist a change in hydrogen-ion concentration in the zones of P_H which are of physiological interest, *i. e.*, P_H 4-10.   In more acid and more alkaline solution this buffering power is weaker and the buffer ratios approximate unity at P_H 3-4 and at P_H 10-11.
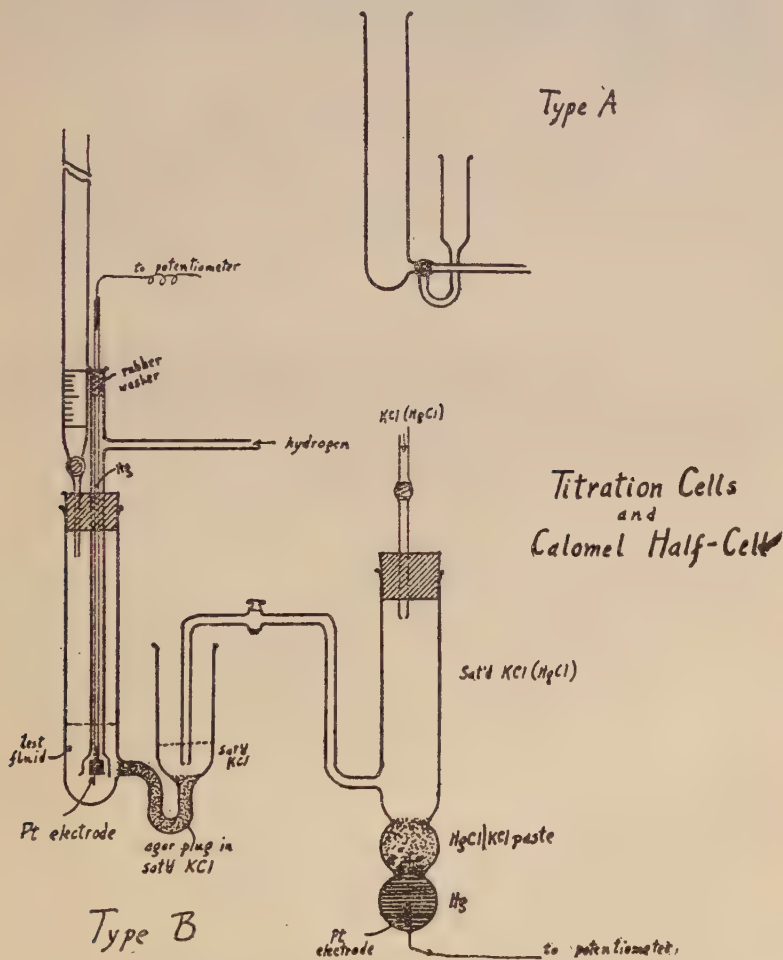
The cells used in conducting electrometric titrations of bacterial suspensions.

2.   Sodium and calcium chlorides, singly and in combination in the concentrations used depress the buffer ratios, particularly in the physiological zones of P_H.

3.   The acidic P_H values at which the buffering power of *Bact. coli* becomes insignificant are approximately those at which this organism is known to be spontaneously agglutinable and to be isoelectric with the menstruum.[1]   It is therefore significant to note that a similar reduction in the buffer ratio is attained at alkaline as well as at acidic reactions.   This observation suggests the existence of a second, an alkaline isoelectric point for bacteria.

212 (2172)

The influence of certain electrolytes upon the electrical charge of bacteria.[2]

By C.-E. A. WINSLOW, I. S. FALK and M. F. CAULFIELD.

[From the Department of Public Health, Yale School of Medicine, New Haven, Conn.]

In connection with an extensive series of studies on the effect of electrolytes upon the various properties of the bacterial cell, we have measured the electrical charge of vegetative cells of *B. cereus* (chosen on account of its large size) by the direct microscopic method described by Northrop.[3]   In conducting these experiments a voltage of known magnitude (112 v.) is applied to non-polarizing zinc-zinc sulphate electrodes and the direction and velocity of migration of the bacteria in unbuffered suspensions determined by observing through the microscope the time taken by the bacterial cells to cross a definite space on the

[1] Michaelis, *Deut. med. Wochensch*, 1911, xxxvii, 969; Eisenberg, *Centr. Bakt.*, 1919, lxxxiii, 70, 472, 561; Northrop and DeKruif, *J. Gen. Physiol.*, 1922, iv, 639.

[2] Studies here reported were aided by a grant from the Loomis Research Fund of the Yale School of Medicine.

[3] *J. Gen. Physiol.*, 1922, iv, 629.

ocular micrometer. In order to avoid theoretical assumptions we have expressed all results in terms of this observed velocity, a high velocity in general presumably signifying of course a greater charge. The average results obtained with varying hydrogen ion concentrations generally based on four or more observations at each P_H value are indicated below.

TABLE I.

Velocity of Migration—Micra per Second.

P_H	1	1.-1.9	2.-2.9	3.-3.9	4.-4.9	5.-5.9	6.-6.9
	—1.2	+0.3	—0.1	—1.8	—7.8	—10.3	—10.3
P_H	7.-7.9	8.-8.9	9.-9.9	10.	11.	12.	13.
	—11.7	—10.8	—12.8	—13.8	—2.2	0	0

It appears that these bacteria maintain a fairly high and reasonably consistent negative charge through the wide range of reaction between P_H 5 and P_H 10, rising somewhat with increasing alkalinity. On the acid side of this range the charge drops steadily to an isoelectric point at about P_H 2.0, the charge beyond this point being slight and variable and sometimes reversed. At reactions more alkaline than P_H 10 the charge diminishes even more rapidly to a second (alkaline) isoelectric point at about P_H 12.

The influence of sodium and calcium salts, alone and in combination, in the concentrations studied, was to depress the velocity of migration very greatly at all P_H values and to narrow the zone of maximum velocity on the alkaline side, bringing the alkaline isoelectric point to P_H 9.0-10.0 (Table II). A solution of 0.145 M $CaCl_2$ is more potent in diminishing velocity than is 0.725 M NaCl and there appears to be an antagonistic effect of the two salts at reactions between P_H 3.0 and P_H 7.0.

TABLE II.

Velocity of Migration—Micra per Second.

P_H	0.725 M NaCl	0.145 M $CaCl_2$	0.580 M NaCl + 0.145 M $CaCl_2$
1.5	—0.8	+1.0	—0.5
3.0	—2.7	—0.8	—2.3
5.0	—3.9	—1.9	—3.5
7.0	—4.9	—3.8	—4.4
8.0	—4.2	—2.6	—2.1
9.0	—0.8	—0.7	0
10.0	0	0	0

In general the curves parallel in a general way those obtained for the viability of bacteria in water, so far as the general influence of electrolytes is concerned except that here antagonism occurs in a more acid zone.   They also correspond well to the observation of Falk and Shaughnessy[1] on the buffering power of suspensions of bacterial cells, particularly in the demonstration of a second alkaline isoelectric point.   In general it appears that within the zone of hydrogen ion concentration which favors the viability of bacteria in water the bacterial cell exerts a high buffering power and maintains a normal electrical charge.   In more acid or more alkaline solutions, or in solution of favorable reaction but in the presence of sodium and calcium salts in toxic concentration, the buffering power fails, the electrophoretic charge is reduced, and the bacteria die.

213 (2173)

The role of phosphate and potassium in carbohydrate metabolism following insulin administration.

By GEORGE A. HARROP, JR., and E. M. BENEDICT.

[*From the Department of Medicine of The College of Physicians and Surgeons, Columbia University, and the Presbyterian Hospital, New York City.*]

Following the administration of large doses of insulin to several diabetics, to two patients with diabetic coma, to a normal fasting individual, to a fasting dog, and to fasting rabbits, and coincident with the drop in the blood sugar which regularly occurs, a marked drop has been noted in the concentration of inorganic blood serum phosphate and serum potassium.   A sharp drop in the urinary output of phosphorus and of potassium accompanies the drop in the blood serum concentration, and is later followed (3-12 hours) by a well marked compensatory increase in the urinary excretion of these substances, so that the total excretion over daily periods is not much altered.

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[1] PROC. SOC. EXP. BIOL. AND MED., 1923, xx, 426.

In sharp contrast to the above findings during the convulsions of insulin shock, a very marked increase in the serum phosphate and serum potassium has been found during strychnine convulsions in rabbits, in which condition, as is well known, an extreme destruction of muscle glycogen occurs.

In seeking an explanation of the above phenomena, attention is drawn to the recent work of Embden, Meyerhof, A. V. Hill, and others, which indicates that a hexose diphosphate is an intermediary between glycogen and lactic acid in the contractile process in the muscles. It is suggested that an analogous phosphate compound is formed during the process of storage of glycogen and that insulin causes or accelerates its synthesis. This would account for the disappearance of phosphate into the tissues during the period in which insulin is acting, and the subsequent increased excretion of phosphate may be due to the further conversion into glycogen of the hexose portion of the hexose diphosphate, thus leaving the excess of phosphate available for excretion. The massive, rapid breaking down of the phosphate compound during the tetanic convulsions of strychnine, would further account for the appearance of inorganic phosphate (as well as lactic acid) in the blood stream, as has actually here been shown to occur. The shift in concentration of the potassium indicates the formation of a monopotassium salt.

214 (2174)

An electrocardiographic sign in pericardial effusion.

By B. S. OPPENHEIMER and HUBERT MANN.

[From the Medical Department, Montefiore Hospital, New York City.]

The electrocardiogram as ordinarily taken is a record of the differences of potential occurring between various parts of the body remote from the heart. While these differences of potential can be shown to be due to a primary electrical effect in the

heart muscle itself, it is evident that they also depend on the nature of the body tissue as a conducting medium. Thus, if the body were composed of a non-conducting material, it is obvious that the primary cardiac electrical effect would not give rise to differences of potential in remote parts, while, on the other hand, if the body possessed the property of a good conductor, such as copper, it can be shown that the difference of potential between parts remote from the heart would be infinitesimal.

These considerations led us to believe that the presence of a large effusion surrounding the heart, especially a large pericardial effusion, might manifest itself by a lowering of the voltage of the electrocardiogram. This belief has been reinforced by our observation of seven cases of low voltage associated with large pericardial or pleuropericardial effusions. In some of these cases the markedly low voltage of the electrocardiogram led to a suspicion of effusion, which was later confirmed by X-ray examination, aspiration or autopsy finding.

The characteristic finding in the electrocardiogram is a decided lowering of voltage of the main deflection in all three leads. This is not necessarily associated with any constant alteration in the shape of the various waves, although it is quite conceivable that the effusion may also alter the electrical axis of the heart in some cases, or cause other changes in the propagation of the electrical disturbance.

It is possible that fairly large effusions may fail to effect the voltage of the electrocardiogram appreciably in certain cases. It is also quite true that some patients have electrocardiograms with low voltage not associated with any effusion, *e. g.,* cases with myodegeneration. However the occurrence of the electrocardiographic finding in fairly constant association with an effusion and the fact that such a relation is to a certain extent predictable in advance, renders it significant and suggests further clinical and experimental observation.

215 (2175)

The hydrolysis of collagen by trypsin.

By ARTHUR W. THOMAS and F. L. SEYMOUR-JONES.

[*From the Department of Chemistry, Columbia University, New York City.*]

The generally accepted statement that collagen is not hydrolyzed by trypsin unless previously swollen in acid or alkali, shrunk in hot water, or treated with pepsin, rests entirely on qualitative work by Kühne and Ewald[1] in 1887 and 1890.

Using finely sifted hide powder as a source of collagen, we found that it was readily digested by trypsin in concentrations of the protease exceeding 10 mg. per liter. The hide powder was treated in 10 c.c. conical graduated centrifuge tubes with buffer solutions at various hydrogen ion concentrations, centrifuged, and the volume of powder measured. The buffer was then replaced by a trypsin solution made up in a buffer of known P_H, and digestion carried on, with continuous shaking, at 40.00°. By suitable variation of the buffer we found that the tryptic hydrolysis was not affected by pretreatment of the collagen at different P_H's between 1.1 and 8.9 and that the optimum reaction for the hydrolysis was at P_H 5.9. The time-hydrolysis curve for trypsin-collagen is of the same nature as with trypsin and proteins in general; since here the substrate is insoluble though hydrated, tryptic action appears to take place at the surface of the substrate particles, whether these be coarsely or colloidally dispersed. The degree of hydrolysis increases with increasing concentration of trypsin and decreasing size of hide powder particles. Complete hydrolysis was not reached in four periods of 20 minutes each, the experiment being then stopped owing to increasing hydrolysis in the control (hide powder and buffer solution without trypsin).

The shape of the hydrolysis-time curve suggests that the reaction is very slightly reversible. Experiments on pelt, using concentrations of trypsin up to 0.4 per cent., showed measurable

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1 *Verh. d. Naturhist. Med. Ver. in Heidelberg*, (N.F.) 1887, i, 451. *ibid.*, (N. F.), 1887, i, 451; *Z. f. Biol.*, (N. F. 8), 1890, xxvi i.

hydrolysis of the collagen, although the organized skin structure inhibited diffusion of the enzyme and greatly decreased the speed of the reaction.

Specimens of collagen tanned with quinone, gallotannic acid, copper sulfate and formaldehyde were all hydrolyzed by trypsin while chrome tanned collagen was not.

216 (2176)

The specific soluble substance of pneumococcus.

By M. HEIDELBERGER and O. T. AVERY.

[*From the Hospital of the Rockefeller Institute for Medical Research, New York City.*]

In 1917 Dochez and Avery[1] showed that there was contained in filtrates from pneumococcus cultures and in the body fluids of experimentally infected animals and of patients suffering from pneumonia, a soluble substance which reacts specifically in anti-pneumococcus serum of the homologous type. This substance, which was found to be thermostable, precipitable by alcohol or acetone, non-dialyzable, and not digested by trypsin, is now being subjected to a more intensive chemical study.

Eight-day, autolyzed cultures of Type II Pneumococcus in phosphate broth were concentrated to 1/15 volume and precipitated with 1.2 volumes of alcohol. The precipitate, centrifuged at high speed, yields a compact middle layer containing the specific soluble substance. By repeated fractionation with alcohol or acetone, first in neutral, then in dilute acetic acid solution, followed by repeated fractional precipitation with ammonium sulfate and final dialysis, about 1 gm. of a highly purified preparation was obtained for each 75 liters of culture used.

In its present state of purity the specific soluble substance is amorphous and yields a viscous solution in water. A 1 per cent. solution gives no biuret test, yields no precipitate with phospho-

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[1] Dochez and Avery, *J. Exp. Med.*, 1917, xxvi, 477.

tungstic acid, mercuric chloride, or neutral lead acetate, gives a faint haze with tannic acid, and is precipitated by basic lead acetate. At a dilution of 1:1,500,000 it still gives the Molisch reaction and yields a precipitate with Type II immune serum. $[\alpha]_D$ is $+58.7°$; N, 1.2 per cent.; P, trace; S, none; C, 46.2 per cent.; H, 6.1 per cent. Hydrolysis yielded 79 per cent. of reducing sugars, of which glucose was identified by the melting point and optical rotation of its phenylosazone. Earlier preparations containing more nitrogen and yielding less reducing sugars on hydrolysis were not specific at as high dilutions.

While it is not excluded that the non-carbohydrate portion of the preparation is actually the carrier of the specific reaction, it is believed that the evidence points to the identity of the specific soluble substance with the polysaccharide portion, thus linking it with the bacterial gums isolated by others from capsular material, but never before connected with specificity.

217 (2177)

Immunological relationships of cell constituents of pneumococcus.

By O. T. AVERY and M. HEIDELBERGER.

[*From the Hospital of the Rockefeller Institute for Medical Research, New York City.*]

In the preceding communication, it has been pointed out that the so-called soluble specific substance of pneumococcus is non-protein in nature, and in its present state of purification is either itself a polysaccharide, or intimately associated with the carbohydrate. Although antigenically this substance appears capable of stimulating little or no antibody response, serologically it is highly reactive and exhibits to an extraordinary degree the reactions of type specificity in antibacterial serum of the homologous type of pneumococcus. On the other hand it is possible to recover from the pneumococcus cell another substance which is protein in character and which is distinctive in its serological behavior from the soluble specific substance. From bile solutions of pneumococci dilute acetic acid precipitates a protein fraction. This precipitate is washed in water and redissolved in dilute al-

kali. After repeated precipitations and thorough washing the dissolved material is passed through a Berkefeld filter and reprecipitated. The final precipitate is washed rapidly with acetone and ether and dried *in vacuo*. The preparation so obtained is a whitish powder, readily soluble in faintly alkaline solution, possessing the properties of a mixture of nucleoprotein and mucoid. It contains about 16 per cent. of nitrogen and 0.5 per cent. phosphorus.

Solutions of nucleoprotein prepared from one type of pneumococcus (Type II) react in about equal degree with all three types of antipneumococcus serum, and not with antityphoid or normal horse serum. This fact, if confirmed by subsequent investigation of the protein from pneumococci of other types, would indicate, on the basis of specific precipitin reactions, that all pneumococci possess in part at least a common specific protein. The protein of pneumococcus, as contrasted with the non-protein fraction or soluble specific substance is not type specific, but reacts with antipneumococcus serum regardless of type derivation. It is therefore species specific, not type specific.

218 (2178)

Gastric antacids which cannot act as systemic alkalies.

By ISIDOR GREENWALD.

[*From the Harriman Research Laboratory, The Roosevelt Hospital, New York City.*]

The antacid most frequently used in the treatment of hyperchlorhydria is sodium bicarbonate. But this is not only an antacid but an alkali, so that the contents of the stomach occasionally become alkaline. Moreover, the amount required to control the gastric symptoms is frequently sufficient to make the urine alkaline. Both these alkalinizations are regarded as unphysiological. Direct evidence of the occasional toxic action of therapeutic doses of sodium bicarbonate has recently become available.[1,2]

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[1] C. A. L. Binger, A. B. Hastings and J. M. Neill, *Arch. Intern. Med.*, 1923, xxxi, 45.

[2] Leo L. Hardt and Andrew B. Rivers, *Arch. Intern. Med.*, 1923, xxxi, 171.

The ideal gastric antacid would appear to be a substance which can neutralize hydrochloric acid but cannot make the stomach alkaline and which is excreted, unchanged, in the intestine and not in the urine. Such substances are found in the secondary and tertiary phosphates of calcium and magnesium. They are neutral substances, which dissolve in the hydrochloric acid of the stomach with the formation of the corresponding acid phosphates. An excess cannot quite alkalinize the stomach. The tertiary phosphates, as was shown by the work of Steel and Gies[1] and of Lothrop[2] for bone-ash, which is very nearly pure $Ca_3(PO_4)_2$, and by experiments of the author (see Table I) for $Mg_3(PO_4)_2$, are precipitated in the intestine and eliminated in the stool. Dogs have received bone-ash, in many times the therapeutic dose, for years without apparent ill effect.

TABLE I.

Effect of ingestion of magnesium phosphate on urinary excretion. Subject G., weight 71 kilos; constant diet, including 1000 c.c. milk daily.

N grams.	P grams.	Ca grams.	Mg grams.	
13.50	1.28	0.253	0.121	
11.20	1.00	0.326	0.156	
13.44	1.25	0.264	0.131	5 grams $Mg_3(PO_4)_2$
12.03	1.40	0.159	0.161	$\backsimeq$ 0.88 gm. Mg)
12.68	1.25	0.182	0.187	with each of 3 meals.

TABLE II.

Neutralizing action of several antacids as determined by adding the amounts shown to 100 c.c. of 0.555 N HCl at 37°, stirring while at 37° for 10 minutes and filtering, if any remained undissolved. The filtrates were used for colorimetric determination of hydrogen ion concentration and for titration with Toepfer's reagent and phenolphthalein as indicators. The results are expressed in c.c. of 0.1 N NaOH required per 100 c.c and as negative logarithms of hydrogen ion concentration.

	$Bi_2(OH)_4CO_3$				"Neutralon"		
Amount grams.	Toepfer.	Phenol-phthalein.	P_H		Toepfer.	Phenol-phthalein.	P_H
0.100	53.0	54.6	1.30		54.3	56.1	1.25
0.200	51.8	52.9	1.30		54.0	56.6	1.25
0.400	50.5	52.2	1.35		51.8	55.9	1.30
1.000	47.2	47.9	1.40		47.2	56.7	1.70
4.000	....	....	....		20.9	53.2	2.00

[1] M. Steel and W. J. Gies, *Amer. Jr. Physiol.*, 1908, xx, 343.

[2] A. P. Lothrop, *Amer. Jr. Physiol.*, 1909, xxiv, 297.

	$Ca_3(PO_4)_2$				$Mg_3(PO_4)_2$	
Amount grams.	Toepfer.	Phenol-phthalein.	P_H	Toepfer.	Phenol-phthalein.	P_H
0.100	44.2	55.4	1.30	45.0	50.6	1.40
0.200	36.4	55.2	1.60	35.0	45.5	1.70
0.400	30.5	54.8	1.80	15.4	37.5	2.30
1.000	9.2	52.6	2.60	—9.1	23.5	6.0
4.000	2.3	51.3	3.30	—16.0	6.0	6.7

	$NaHCO_3$		
Amount grams	Toepfer	Phenol-phthalein	P_H
0.100	44.8	46.1	1.43
0.200	32.6	35.6	1.51
0.400	7.8	14.8	1.95
1.000	—65.0	—2.9	8.4

	$CaCO_3$		
0.100	36.2	37.4	1.45
0.200	16.4	19.0	1.65
0.400	—6.1	0.4	7.0
1.000	—7.6	—0.4	7.0

	M_gO		
0.100	13.0	14.3	1.9
0.200	—2.4	—0.4	8.0
0.500	—4.3	—2.3	9.1

Calcium phosphate is slightly constipating and magnesium phosphate is slightly laxative. The tendency of the patient to constipation or to diarrhea determines the nature of the compound to be used. The dose required is from one to three grams.

The use of calcium carbonate and of magnesium oxide probably tends to a slight alkanization of the system, the degree depending upon the nature of the diet. As against $CaCO_3$, the use of the phosphate has the added advantage of avoiding belching and as against MgO, of avoiding alkalinization of the stomach, for MgO may produce a reaction as alkaline as P_H 9°.

Bismuth subcarbonate and "neutralon" have comparatively little power to neutralize hydrochloric acid in the concentration found in the stomach. Their therapeutic action probably depends upon other factors.

Calcium and magnesium phosphates have been used by several physicians for from a few months to more than two years, with favorable results in most cases. Some patients do not obtain relief. In these, it is probable that a systemic alkanization,

the liberation of a large quantity of gas or some other effect is required.

I am indebted to Dr. John L. Kantor for his assistance in obtaining clinical material and for his careful observation of the patients. He is preparing a clinical report.

219 (2179)

Factors involved in blood volume regulation.

By ALFRED CHANUTIN (by invitation).

[*From the Sheffield Laboratory of Physiological Chemistry, Yale University, New Haven, Conn.*]

That the volume of the blood is not normally constant is shown by distinct diurnal variations in this value as determined by the hemoglobin percentage when the animal (dog) is in complete muscular rest and unanesthetized. Administration of ether is immediately followed by a decrease in the relative blood volume which condition persists not only throughout the period of anesthesia but for some time afterward.

When isotonic saline is injected intraperitoneally the diluting effect on the blood volume is not observed until several hours later. The oral administration of saline dilutes the blood to a greater degree in similar time than similar doses of water. As might be expected, intravenous injection of saline show the most pronounced degree of dilution. Usually, however, the normal relative blood volume is attained after approximately two hours following the intravenous injection (100 c.c. per Rg. body wt.). At this time, when large volumes of fluid are unaccounted for, neither the muscles nor the liver show a detectable increase in fluid-not-blood. Renal activity is not essential for this prompt adjustment of blood volume for the urine volume does not account for the "lost" fluid.

220 (2180)

Diet and tissue regeneration.

By ARTHUR H. SMITH and T. S. MOISE.

[*From the Sheffield Laboratory of Physiological Chemistry and the Department of Surgery, Yale University, New Haven, Conn.*]

Two types of diets were used: one, containing casein as the protein, and being adequate for normal growth; the other, having gliadin as the protein, sufficing for maintenance but not for growth. Both foods provided 5.3 Cals. per gram and were identical in their composition except for the difference in quality of the protein.

White rats of similar ages were employed. A standard tissue damage (liver necrosis) was induced by subcutaneous injection of 1 c.c. sterile mineral oil containing 0.15 c.c. chloroform per 100 grams body weight, which had been previously determined to be the maximum non-lethal dose. The animals were killed at definite intervals, a control rat on standard food together with an experimental rat on gliadin food.

This procedure produces a necrosis of the liver cells around the central vein of the lobule appearing at its maximum 24 to 48 hours after the injection. In both series of rats there occurs an early mobilization of leucocytes and clearing away of the cellular debris with a simultaneous initiation of regeneration from the uninjured cells at the periphery of the lobule. The process of repair is most active at about 72 hours after the injection in both series. At 120 to 148 hours the regeneration is apparently complete and the liver is histologically normal.

The rate of procedure of the repair, so far as we have been able to observe, is the same for both groups of animals. This fact raises the question as to the possibility of such repair involving, as it does, tissue reconstruction upon the gliadin food— a diet upon which general body growth is impossible. A discussion of the application of these results to the theories of intermediary protein metabolism will be reserved for a later publication.

221 (2181)

The effect of extirpation of the uterus on the life and function of the corpus luteum in the guinea pig.

By LEO LOEB (by invitation).

[*From the Department of Comparative Pathology, Washington University, St. Louis, Mo.*]

It is usually assumed that extirpation of the uterus is without any noticeable effect on the ovaries, outside perhaps of cystic degenerative changes which have sometimes been observed to follow this operation and which evidently are due to interference with the blood supply of the ovary following injury of the uterine vessels.

Experiments in the guinea pig have however convinced us that a complete or almost complete extirpation of the uterus may be followed by a very characteristic effect, namely a long continued preservation and function of the corpus luteum. Instead of beginning to degenerate fourteen or fifteen days after ovulation, the corpus luteum may remain well preserved and even show attempts at mitotic proliferation for sixty days, or perhaps even as late as eighty days after the last heat. We have not yet determined the limit of preservation of the corpus luteum under these conditions. The cyclic corpus luteum thus equals or perhaps surpasses in vitality the corpus luteum of pregnancy which latter has in the guinea pig a duration of about sixty-five days.

In order to demonstrate this effect we extirpated the uterus a few days after ovulation. The corpus luteum which develops as a result of ovulation remains preserved for a long time following this operation. If we extirpate the uterus in very young guinea pigs at a time when an ovulation has not yet occurred, the first ovulation takes place, notwithtanding the extirpation of the uterus. In various experiments we observed this ovulation to occur as early as nine days and as late as twenty-nine days following the operation. The corpus luteum which thus originates remains then preserved for a long period of time and mitotic figures may often be seen in the lutein cells. In ovaries of these young animals, which ovulated for the first time fol-

lowing hysterectomy, there is usually no remnant of retrogressing corpora lutea representing former ovulations visible. In one case, however, we found such a body side by side with a well preserved corpus luteum. This was probably due to the fact that one of the corpora lutea formed during an ovulation may retrogress earlier than the others.

We see then that extirpation of the uterus does not prevent ovulation as such, but that it has such an effect only indirectly by preserving the life of the corpora lutea. Also in other respects the ovaries in hysterectomized guinea pigs are normal. Follicles grow in the normal manner, mature and undergo atresia. The maturation of follicles is not interfered with under those conditions.

These corpora lutea not only live, but they also function. They prevent the occurrence of an ovulation during the whole period of their life; but if the corpora lutea are extirpated completely, a new ovulation takes place at an early period notwithstanding the absence of the uterus. This is an additional proof that the hysterectomy as such does not prevent ovulation. On the other hand, if we prevent the maturation of follicles through underfeeding, a new corpus luteum does not develop in a young guinea pig, which has not yet ovulated, even after extirpation of the uterus.

This marked prolongation in the life and function of the corpus luteum may not only be obtained after a complete hysterectomy, but even in cases in which a very small amount of uterine tissue has been left back, an amount sufficient to give rise to the development of a placentoma. In such a case we observed as late as forty-six days after ovulation mitotic proliferation in placentomatous tissue without the presence of pregnancy.

In these hysterectomized animals the mammary gland develops quite markedly and mitotic proliferation may be observed in such glands as late as seventy-four days after the last ovulation. In one case we found sixty days after ovulation milk production in a mammary gland which had previously proliferated. During the prolonged period of function of the corpus luteum prooestrus and oestrus are prevented and thus growth processes do not occur in the vagina. The epithelium of the vagina becomes therefore very vacuolar and polynuclear leucocytes migrate through the mucosa into the lumen in hysterectomized guinea pigs.

If a slightly larger part of the uterus is left back—perhaps one-third or one-fourth its length—the full effects of this operation are not observed, although the life and function of the corpus luteum is somewhat prolonged even under these conditions. In different experiments the ovulation following an incomplete extirpation occurred between a period of twenty-one and thirty-two days following the preceding ovulation, but a delay in the later ovulations was either absent or only very slight. A relatively small part of the uterus can therefore, at least to a great extent, take over the function of the whole uterus. The prolongation of the life of the corpus luteum which occurs even under these conditions may again call forth a proliferation in the mammary gland in some cases. However, there are certain factors in the growth of the mammary gland which need still further investigation.

We believe that this method of prolonging the life and function of the corpus luteum will prove of value in the analysis of the mechanism of the sexual cycle.

Differences in the effect of hysterectomy in different species depend presumably on differences in secondary factors; it is not probable that in principle the effect of the uterus on the corpus luteum differs in different species.

222 (2182)

The mechanism of the sexual cycle and the specificity of growth substances.

By LEO LOEB (by invitation).

[*From the Department of Comparative Pathology, Washington University, St. Louis, Mo.*]

The experimental analysis of the sexual cycle carried out particularly during the last fifteen years makes it possible to state the main factors regulating its mechanism. While this analysis rests largely on experiments and observations in rodents, and especially in the guinea pig, yet in principle conditions seem to be similar in all the mammals.

Two phases can be distinguished in the sexual cycle; the first phase is dominated by an ovarian factor other than the corpus luteum, in all probability by the maturing follicles the wall of which secretes a substance which causes various kinds of circulatory changes and growth processes and in addition certain psychical alterations. Proliferation, under the influence of this substance, occurs in the mammary gland, in the vagina and also in the uterine wall; this substance calls forth changes in the ovary which culminate in ovulation.

These growth processes usually cease in the vagina suddenly with the appearance of oestrus, while in the mammary gland they may continue for a short time longer.

Ovulation leads to the formation of the corpus luteum. The second phase of the sexual cycle is dominated by a substance or substances given off by the corpus luteum. This substance or these substances sensitize the uterus, making possible the production of decidua or of placentomata, or the normal predecidual proliferation and facilitating the fixation and development of the fertilized ovum; they cause growth processes in the mammary gland and prevent procestrus, oestrus and ovulation; the corpus luteum on the other hand does not prevent the maturation of follicles, at least in rodents. The corpus luteum substance is without a direct effect on vagina, tube or other connective tissue or epithelial structures of the guinea pig. This substance or these substances act on different tissues at different periods of the sexual cycle. The sensitization of the uterine wall is limited to the first half of the life of the corpus luteum. The growth of the mammary gland occurs in the rabbit during the whole period of the life of the corpus luteum; in the guinea pig it begins about on the sixteenth day of the cycle. The substance inhibiting the effects of the maturing follicle acts throughout the whole period of the preservation of the corpus luteum. When degenerative changes occur in the corpus luteum, this function ceases.

Between these two phases, the first phase probably dominated by the maturing follicle and the second, the lutein phase, there may be a short intermission during which the first substance has ceased to act and the second substance has not yet been produced in sufficient quantity to be effective. Certain degenerative processes may occur during this intermission, caused probably

by acute circulatory disturbances and by secondary destructive effects of leucocytes.

We would conclude on the basis of the observations of Long and Evans in the rat that in this species the sexual cycle consists essentially only of the first phase and of the intermission; the lutein phase is here lacking under ordinary conditions, but can be called forth experimentally and is present also during lactation. We would interpret in a similar way the observations of Allen in the mouse.

The specific relation of these growth substances to certain tissues is very pronounced. The corpus luteum substance of the guinea pig acts very strongly on the connective tissue of the uterus, but not at all on that of the vagina; the follicular substance acts on the vagina and on the uterus in a manner quite different from the corpus luteum substance. This specificity is due to the character of these growth substances as well as of the tissues on which they act, and not to the position of the tissues of the body. It can be shown that this specificity is still noticeable in uterine tissue after it has been transplanted subcutaneously.

On the basis of these observations we would propose the following classification of the periods of the sexual cycle. In the various species individual differences exist; thus in the rat and mouse only phases I and II can be recognized.

I. Follicular phase—
    a. Proœestrus.
    b. Oestrus.
    c. Metoestrus (in some cases).

II. Intermediate phase (following ovulation and preceding sentization of the uterus).

III. Lutein phase—
    a. Period of sensitization of uterus.
    b. Period of return to the resting state.
    c. Period of resting state.

223 (2183)

Types of mammalian ovary.

By LEO LOEB (by invitation).

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We can distinguish among mammals at least three types of ovaries. They are represented among the rodents by the ovaries of the guinea pig, rat and rabbit.

(1). In the first type, that of the guinea pig, in the œstrus period, preceding ovulation by a number of hours, the large majority of all follicles—all but the very small ones—begin to degenerate. In the larger follicles the granulosa becomes karyorrhectic en masse and connective tissue begins to grow into the follicular cavity after the destruction of the granulosa epithelium has progressed still further. In smaller follicles the process of destruction of the granulosa is less obvious and the granulosa cells disappear and here also connective tissue soon begins to invade the cavity. In the week following ovulation the very small follicles develop again and pass through their cycle; about eight days after ovulation the follicles have again reached full size and maturation on the one hand and atresia on the other hand can set in; this atresia usually affects only the large follicles.

(2). In the second type, that of the rat, ovulation is not preceded by a general atresia of follicles, but the large follicles mature, rupture and become converted into corpora lutea. There takes place throughout the cycle a limited atresia of follicles. In the rat the ingrowth of connective tissue into the atretic follicles is less active than in the guinea pig, in consequence of which the cavity of the degenerating follicle remains preserved during a longer period of time in the former species. In both guinea pig and rat ovulation is spontaneous. It occurs as soon as follicles have had a chance to mature and the inhibiting influence of the corpus luteum has ceased to exert itself. In the guinea pig a considerably longer period is necessary for the maturation of the follicles than in the rat, because in the former additional time is needed to allow follicles to develop from very small to large size, while in the rat only the very large follicles are destroyed at the time of ovulation and therefore maturation

of a new set of follicles can take place in a very short time. Inasmuch as also the functioning period of the corpus luteum is shorter in the rat than in the guinea pig and degeneration sets in much more rapidly in the former, the sexual cycle is much shorter in the rat than in the guinea pig.

(3). In the third type, that of the rabbit, not only does an atresia of follicles en masse not occur before ovulation, and thus less time is required for the production of new mature follicles after an ovulation has occurred, but in addition spontaneous ovulation does not occur in the rabbit; a copulation is usually required to call forth an ovulation. Thus in the female rabbit separated from the male instead of a rupture of the mature follicles an atresia of the large follicles occurs; in addition small follicles may also become occasionally atretic. In consequence of the absence of a spontaneous ovulation in the rabbit the second phase of the sexual cycle, that dominated by the corpus luteum, is absent in the female rabbit kept separated from males; but it is present after sterile copulation. It is normally present in the isolated female guinea pig and is absent in the rat on account of the shortness of the sexual cycle. In the mouse, according to E. Allen, spontaneous as well as non-spontaneous ovulation occurs.

Associated with these differences in the ovarian structure and function are differences which concern especially the development of the interstitial tissue. In the rabbit the thecæ internæ of atretic follicles show a considerable enlargement; at the same time they almost assume the character of a gland like structure; or of connective tissue under special conditions, namely when it organizes material which the connective tissue cells can phagocytize: thus a tissue originates consisting of large cuboidal cells, closely joined together. In the guinea pig the theca interna cells of atretic follicles remain on the whole small and do not in the least become similar to gland like structures. In the ovary of the rat the development of the theca interna has a character intermediate between that of the rabbit and and the guinea pig.

It can be understood that in view of the en masse occurrence of follicular atresia in the guinea pig and the subsequent development of later stages of atresia simultaneously in many follicles, sufficient space for the gland like development of interstitial tissue may not be available in the guinea pig. In the rabbit and rat where only isolated follicles degenerate the thecæ internæ

should have a better chance to expand and accordingly we find a greater development of the so-called interstitial gland in these species; it is most pronounced in the rabbit, where a periodic spontaneous formation of cyclic corpora lutea is lacking, and where thus the greatest amount of space is available for the development of an interstitial gland. It is therefore possible that the character of the interstitial tissue in the mammalian ovary is at least partly determined by mechanical factors more or less favorable to the expansion of the theca interna cells of atretic follicles.

It is also of interest to note that in the guinea pig the number of follicles rupturing at the time of ovulation is usually much smaller than in the rat and in the rabbit.

These differences between these species may perhaps depend upon a different degree of sensitiveness of the granulosa of the follicles. It is apparently greatest in the guinea pig, while the follicles are more resistant in the rabbit and rat. In all these species the sensitiveness of the follicles increases with increasing size, until maturing has been reached, when with the cessation of cell proliferation and an increase in cytoplasm a great increase in resistance seems to occur, which makes it possible for the mature follicle to withstand those influences which in other follicles cause atresia. This increase in resistance in the mature follicles makes ovulation possible. On the other hand the ova, which in all probability determine the development of the follicles (Loeb, Walsh), are at least as resistant in the guinea pig as in the rabbit and in the rat, and in the first species they tend apparently more strongly to progressive parthenogenetic changes within the ovary than in the rabbit and rat.

It would be of interest to study the ovaries of other mammalian species from this point of view, in order to determine which of these relationship are constant and which are variable among the various factors which we considered.

224 (2184)

Concerning the detection of pentose, formaldehyde and methyl alcohol.

By JAMES B. SUMNER.

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Bial's reagent, as ordinarily made up, does not keep. It is better to dissolve the orcinol and ferric chloride in alcohol, (6 gm. orcinol, 40 drops ferric chloride in 200 c.c. alcohol). Heat 5 c.c. of sugar solution with 15 drops of the above and an equal volume of fuming hydrochloric acid. A blue color develops if pentose is present.

When formaldehyde is subjected to this test a white precipitate is formed if 0.1 mg. per c.c. is present. Upon heating the precipitate turns brown. Acetaldehyde gives a precipitate which does not turn brown. With minute quantities of formaldehyde there is no precipitate but if the test is made strongly alkaline a strong green fluorescence is observed.

Orcinol can be used to detect methyl alcohol as follows:

Distill off the alcohol, using a Vigreux column and allowing as little water to pass over as possible. Place 1 c.c. of the distillate in a large hard glass test tube, which is free from scratches. Add 2 c.c. of 6.7 per cent. potassium dichromate solution and 2 c.c. of 62 per cent. sulfuric acid. Mix at once and let stand for 10 minutes. Add 15 c.c. of distilled water, mix and heat in boiling water for 10 minutes. Add 1 c.c. of a 0.5 per cent. aqueous solution of orcinol. Mix well and continue the heating for 30 minutes. If the original alcoholic distillate contained 5 per cent. of methyl alcohol a heavy white precipitate will appear after 5 minutes heating. With 0.5 per cent. methyl alcohol a faint precipitate is formed after heating for 30 minutes, or upon cooling. To show smaller quantities of methyl alcohol add a slight excess of sodium hydroxide, heat for a few minutes and filter. The filtrate will show a green fluoresence if traces of the methyl alcohol were present. Formic acid, acetone, furfurol, amyl alcohol and acetaaldehyde do not interfere with this test. Glycerine interferes if added to the distillate; acrolein interferes if present in considerable amount.

225 (2185)

The vitamin content of raisins, dried raisin seeds and raisin seed oil.[1]

By R. ADAMS DUTCHER and JULIA OUTHOUSE.

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Commercial raisins are most commonly prepared by drying two types of grapes, the small Sultanina (Thompson seedless raisin) and the large Muscat of Alexandria (commonly called the Muscat or Malaga raisin). When the latter is dried and sold without removing the seeds, it is known as "loose" Muscat raisin. When the seeds are removed the term "seeded Muscat raisin" is applied.

In this investigation studies have been made on the Thompson seedless raisin, the loose Muscat raisin, the seeded Muscat, raisin seed oil and dried raisin seeds. One hundred and fifty-six rats were used in the experiments dealing with the study of vitamin A and the usual technique was employed with the exception that the yeast was fed in pellet form separate from the ration. In all of the experiments the animals were confined in separate cages and at least eight animals constituted an experimental groups, on the same diet. Food intake records were kept for each individual. The raisin materials were mixed (in ground form) with the basal ration, with the exception of the raisin seed oil, which was fed separately.

A total of eighty-three rats were used in the study of vitamin B and the usual technique was followed with the exception that 5 drops of crude cod liver oil were fed separately each day instead of butter fat.

Thirty guinea pigs were fed the following basal ration in the study of vitamin C.

Rolled oats	40 per cent.
Milk powder	30 per cent.
Bran	19 per cent.
NaCl	1 per cent.
Yeast	5 per cent.
Cod liver oil	5 per cent.

The oats, milk powder and bran were mixed and autoclaved for two hours at a pressure of twenty pounds and the other ingredients were added later. Inasmuch as the guinea pigs would not eat the raisin preparations a water extract was prepared daily in a standardized manner.

The results obtained indicate that

1.   There is little, if any, vitamin A in Thompson seedless raisins, loose Muscat raisins, seeded Muscat raisins or raisin seed oil.

2.   Vitamin B is present in the Thompson seedless raisin. When forty per cent. of the basal ration consisted of Thompson seedless raisins rats grew normally.

3.   Loose Muscat raisins (with seeds) appear to be richer in vitamin B than the Thompson seedless raisin, good growth having been obtained on a basal ration consisting of twenty per cent. of loose Muscat raisins.

4.   Seeded Muscat raisins seem to have about the same content of vitamin B as Thompson seedless raisins.

5.   Dried raisin seeds were not as rich in vitamin B as Thompson seedless raisins.

6.   It is possible that a portion of vitamin B is destroyed during the steaming, seeding and subsequent drying process in the manufacture of seeded Muscat raisins.

7.   It was not possible to demonstrate the presence of vitamin C in the Thompson seedless raisin, the loose Muscat raisin or the seeded Muscat raisin.

A detailed report of this work will be published later.

226 (2186)

Kidney hypertrophy produced by diets unusually rich in protein.

By THOMAS B. OSBORNE, LAFAYETTE B. MENDEL, EDWARDS A. PARK and D. DARROW.

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In preliminary reports[1] Osborne and Mendel demonstrated that rats can grow to considerable size on diets consisting of nine-tenths or more of protein, provided that they receive a suitable supply of vitamins A and B as well as of inorganic salts. Both casein and washed meat were used as the sources of the protein. Similar tests have since been made with rations containing about 75 per cent. of protein in the diet. It seemed unlikely that rations on which young rats grew from 60 to 260 grams could be extremely harmful to the organism. However Squier and Newburgh[2] have concluded, in harmony with a widespread popular belief, that "a high protein diet in man is a renal irritant"; and Newburgh and Clarkson[3] have described the production of arteriosclerosis in rabbits on "diets containing 27 and 36 per cent. of protein derived chiefly from beef." For this reason it seems worth while to give a preliminary account of our observations on some of the organs of rats growing on our diets very high in protein. ✓

The only striking change was found in the kidneys, which in the animals on the high protein diets were greatly hypertrophied. The average weight of the kidneys was almost twice that of the kidneys of control animals and their size about one-third greater. Microscopic examination showed no changes of an inflammatory or degenerative nature. The exact histological condition of the kidneys and of the other organs will be reported in full in a

[1] Osborne, T. B., and Mendel, L. B., Proc. Soc. Exp. Biol. and Med., 1921, xviii, 167; *Proc. Nat. Acad. Sc.*, 1921, vii, 157.

[2] Squier, T. L., and Newburgh, L. H., *Arch. Int. Med.*, 1921, xxviii, 1.

[3] Newburgh, L. H., and Clarkson, S., *Jour. Am. Med. Assn.*, 1922, lxxix, 1106.

subsequent paper. Hypertrophy of the kidneys existed without hypertrophy of the heart. The ratio of the weight of the heart as well as of the liver to the body weight was about the same in the animals fed the high protein diets as in the control animals. The ratio of the weight of the kidneys to the body weight in the animals on the high protein diets was, on the average, almost double that of the control animals. The hypetrophy occurred whether the protein used was of animal or vegetable origin or was rich or poor in phosphorus.

The animals on such diets were poorly or, at best, only moderately well nourished. The subcutaneous fat was scant and the skin adherent. There was some fat in the abdominal cavity and in certain animals it was fairly plentiful, but in none was it so abundant as in the control animals. In a considerable proportion of the animals the lungs showed the infection so commonly seen in the domestic rat. The thymus was invariably atrophied. The heart was normal. The spleen varied greatly in size. In some rats it was large, in others normal, and in others atrophic. The liver presented no gross abnormalities. The testes in some of the animals were normal in size; in others they were exceedingly atrophic.

227 (2187)

A contribution to the bio-physics of intestinal absorption.

By J. S. VAN DER LINGEN and D. I. MACHT.

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The following experiments were suggested by purely bio-physical considerations concerning absorption from the intestines and it was deemed desirable to report the same in this preliminary communication.

On purely physical-chemical grounds it can be shown that the absorption of a chemical in solution which flows through a membranous tube will depend on the speed of flow of the solution through the lumen of the tube. The authors attempted to

test this postulate on the living intestine in the following manner. Loops of intestines were tied off in dogs, cats and rabbits under anesthesia, and large inflow and outflow cannulas were attached to the two ends of the same respectively. A solution of the drug was perfused through the loop under proper precautions from above downwards, maintaining the temperature of the animal constant and a record was made of the blood pressure and respiratory effects. The drugs employed in these experiments were solutions of potassium cyanide and the powerful alkaloid, aconitin. These solutions were perfused at different rates as indicated by the outflow per minute. It was found that when the solution was perfused with slow speed the absorption was much greater than when the rate of flow was more rapid. The drugs may be perfused so rapidly that very little or no absorption of the poisons takes place at all. As illustrations of the above may be given experiments performed on two cats on December 21st, 1921.

Each animal was anesthetized with ether and in each after laparotomy a loop of intestine 30 cm. long was tied off. Through one of these a solution of potassium cyanide 0.5 per cent. was perfused, at the rate of 100 c.c. per minute. In the other cat a solution of the drug in the same concentration was perfused at the rate of 500 c.c. per minute. It was found that the first signs of poisoning as indicated by the respiration and blood pressure curves in the first cat came on much earlier than in the second cat and death occurred in the first experiment in less than one-half of the time after which it occurred in the second experiment. These results could be only interpreted on the assumption that absorption took place much more rapidly in the first cat than in the second. In another experiment, a dog was used and a loop of intestines of the animal was perfused with potassium cyanide, 1 per cent. solution. When the rate of perfusions was 200 c.c. per minute distinct signs of absorption were noticed as indicated by the respiration and circulation. When the rate of perfusion was increased to 700 c.c. per minute these signs disappeared and apparently very little absorption took place. Similar experiments were made in dogs and cats with preparations of potassium cyanide and also of aconitin. The results of these experiments indicated that the absorption was much more rapid when the rate of flow through the intestines was slow.

In another series of experiments, the authors put to test a different idea. It was thought interesting to determine in which way and to what extent intestinal peristalsis would influence the absorption of drugs from closed loops of intestine. To determine this point, experiments were made with the dye, phenolsulphonphthalein. A loop of intestine was tied off in a cat under anesthesia and 1 c.c. of phenolsulphonphthalein diluted in 10 c.c. of water was injected into the same. At the end of one hour the animal was killed, the intestinal loop was excised and the dye remaining therein was carefully rinsed out with alkaline solution and the amount of drug quantitatively determined by colorimetric methods. Another cat was treated in the same way. An intestinal loop of the same length was made, the same amount of dye and water was injected, but in addition to this the animal was given a hypodermic injection of a small dose of pilocarpin in order to stimulate intestinal peristalsis. At the end of one hour the amount of dye in the intestines and hence, the amount of dye absorbed was determined as above. In a third cat a similar experiment was performed but in this cat intestinal peristalsis was inhibited by an injection of atropin and after one hour, the amount of absorption was again colorimetrically determined. It was found that after mild stimulation of intestinal peristalsis, as for instance with small doses of pilocarpin (0.2 mgm. per kilo) absorption also was greater than in a normal cat. On the other hand, after inhibiting the peristalsis with atropin or with other drugs, absorption was not as great, as illustrated by the following experiments.

Cat A. Loop of 30 cm. injected, 1 c.c. of phenolsulphonphthalein in 10 c.c. water. Absorption at the end of one hour, 55 to 60 per cent.

Cat B. Intestinal loop of the same length and the same amount of phenolsulphonphthalein was injected. The cat was given a hypodermic injection of pilocarpin hydrochloride (0.2 mgm. per kilo). At the end of one hour the amount of dye absorbed was 80 to 90 per cent.

Cat C. Similar loop of intestines was injected with the same amount of dye. Atropin sulphate, 1 mgm., was given to this cat. Amount of dye absorbed at the end of one hour was 40 per cent.

In another series of experiments the intestines were paralyzed by an intraperitoneal injection of a 1 per cent. solution of benzyl

alcohol which inhibited peristalsis by direct action on the muscle cell.   The same results as with atropin were obtained in this cat.   In the case of pilocarpin, the increased absorption is to be explained by more violent peristaltic movement of the intestinal muscles which knead the intestinal contents more vigorously and in that way promote more rapid absorption of the dissolved substances.   When a large dose of pilocarpin was injected, for example, 1mgm. or more per kilo, the intestines went into a tonic spasm and this clamping down of the intestinal muscles decreased absorption to below the normal.   Further studies are in progress, especially in regard to the relation of blood circulation to absorption from intestines.   This communication is in the nature of a preliminary report and it is hoped that further work may be continued on the subject.

228 (2188)

Effects of repeated transplantation of whole suprarenals into young doves.

By OSCAR RIDDLE and TADACHIKA MINOURA.

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In much of the literature on the suparenal glands one or another relation of these organs to sex and to reproduction is more or less clearly indicated.   In a study of these relationships only slight success has been obtained from efforts to transplant suprarenal tissue. [1,2]   The present study was chiefly an attempt to obtain functional grafts of whole suprarenals in young doves and pigeons; or, if persistent transplants were not obtained, to repeat the transplantations at intevals during growth so that some of this tissue would be either functioning or in course of resorption during a considerable part of the period of immaturity; and then to examine several aspects of sex and of reproduction in the treated animals.

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[1] Poll H., *Arch. f. mikr. Andt.*, 1899, liv, 440.

[2] Schmeiden, F., *Zeitschr, f. Chirugie*, 1903, lxx, 453.

We have failed in our attempts to obtain permanent grafts. A number of eggs were obtained from each of the transplanted doves and offspring of these birds were reared. Records were made for both the parents (transplanted) and offspring during a period of 30 months and data obtained for various reproductive capacities and characteristics (age of maturity, fertility, viability, rate of egg production, number and kind of various abnormalities). An examination of these data indicates that none of these characteristics in the offspring can be associated with the transplantations made upon their parents; and that most of these reproductive characteristics were also unaffected in the transplanted birds themselves. This considerable amount of data comprising negative results need not be further considered and is left with the above brief statement. It is thought, however, that two or three by-products of these transplantation tests are of sufficient interest to record, though the data are too meagre to warrant conclusions.

Fifteen ring doves and five common pigeon young received the first transplants at 5 to 10 days after hatching (age was therefore 19-24 days). Additional transplants were made in several cases at about 25, 50, 75 and 100 days later. Pairs of suprarenals taken from related young birds—often from brother or sister—were used in each graft. In the first and second grafts upon each bird one whole gland was placed subcutaneously in the axilla and another on the featherless area of the breast, where the very thin and quite transparent skin permitted easy and frequent observation on the condition of the graft. Birds grafted a third, fourth and fifth time received both glands at slightly separated points on the breast area. Although several of these grafts plainly obtained a blood supply, and gave the *appearance* of remaining essentially intact for periods up to 10 days, our superficial observations together with the three grafts removed at various intervals for sectioning indicates a practical failure of the grafts. A temporary or partial functioning of the grafts cannot of course be excluded; their resorption may be looked upon as in some respects equivalent to the injection of macerated suprarenal tissue.

Omitting all reference to the smaller group of common pigeon young thus transplanted we have summarized in table I certain data obtained from the ring doves. Only 11 of the 15 grafted

birds were healthy when killed for examination at 7 to 12 months old, and only healthy birds are usable in the comparisons made in this table. The tabulation indicates that the size of the suprarenals of the grafted birds (10.2 mgms. for females; 9.8 for males) was not measurably different from that of the control (10.3 for females; 10.6 for males). The body weight of the transplanted birds was somewhat smaller, for both males and females, than was that of the control. The significance of this difference is questionable; the data can be of value only in connection with further results obtained on other animals.

The data obtained for the age of maturity and for gonad size in the birds receiving the transplants are of greater interest on account of their bearing on the view of Varaldo,[1] Krabbe,[2] and others, concerning an antagonism between the suparenal cortex and the sexual glands of the female. The few data of table I indicate that the ovaries of the transplanted birds were smaller than the ovaries of the control (169 mgms. and 237 mgms.), while the testes of transplanted birds seem larger than those of the control (1070 and 706 mgms.). It must be said, however, that the variability is great, that the individual differences show no relation to the number of grafts made, and that our data standing alone are quite inadequate. In fact our complete data indicate that the transplanted males probably did not develop *functional* sperm as early as the transplanted females produced eggs—nearly all of the earliest eggs produced being infertile, though the operated males and females were kept constantly together. Even this latter observation is further complicated by the fact that this group of females showed a strong tendency to mate with *females,* and with the further fact that their eggs were produced at a very early age.

The early maturity of the transplanted females is perhaps the point of greatest interest in the data of table I. These birds produced their first eggs at an average age of less than 157 days. Less than 157 because one of four birds (Nos. 1, 3, 6 or 7) produced a single egg at an earlier age than that given in the table. The mothers of these birds produced their first eggs at an average of 204 days; but this comparison with their daughters is not entirely fair since these mothers were not killed at early

[1] Varaldo, F. R., *Zentralb Gynakol.,* 1913, xxxvii, 1350.
[2] Krabbe, K. H., *New York Med. Jour.,* 1921, cxiv, 4.

age and the unhealthy individuals eliminated from the list as was done for the daughters. Other factors recognized by us are also involved; but we know from much other work that although there is a wide individual variation the normal age of maturity for groups of these doves lies above 160 days. It is almost certain that the transplants did not retard the period of sexual maturity in the females; and, if the extreme tameness which developed from the repeated handling of these birds could be eliminated as a factor (which we can not now do) it would become highly probable that the repeated transplantations accelerated the attainment of sexual maturity in the females.

The data of table 2 indicate that, as administered by us, four injections of fresh suprarenal tissue of the ox into young ring doves did not give results comparable with those from the above transplantation tests. After these injections the growth curve was normal or nearly normal for both males and females. The three surviving males attained and maintained body weights of 166-192 grams (ave., 177); the four females, 151-173 grams (ave., 158). All of the four tested females matured perhaps later than is normal, the earliest at 208 days and the group at an average of 260 days.

A water soluble extract of ox cortex is reported by van Herwerden[1] to have shown favorable effects on health and growth of Daphnia, Limnea and tadpoles. Our four injections of this tissue into doves during a 10-day period would not adequately test its effect on growth; but that such administration of ox suprarenal does not produce the same results on the age or time of female sexual maturity as does transplantation of the suprarenals of closely related individuals, is indicated by the data obtained by us. Obviously there were differences in both the source and the quantity of the tissue absorbed; and an extreme difference in the rate of absorption of the injected and grafted tissues. If, however, the transplanted tissue functioned during several days it may have exercised much the greater action.

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1 v. Herwerden, M. A., *Biol. Zentralb.*, 1922, xlii, 109.

TABLE 2.

Data from young ring doves given four subcutaneous injections (40 to 100 mgms. each) of fresh ox suprarenal tissue within a 10-day period.

Bird			Remarks
No.	Sex	Age (mo.) at first injection	
			Injections of fresh suprarenal cortex
25	♂	3.8	Killed at 12.1 mo.; tuberculous; normal growth and behavior.
26	♀	3.6	Died at fourth injection; reproductive organs unaffected.
27	♀	3.0	Killed 4 days after fourth injection; reproductive organs unaffected.
28	♀	3.0	Killed 4 days after fourth injection; reproductive organs unaffected.
29	♀	1.8	First egg laid at 244 days; mother's first egg at 212 days.
30	♀	1.7	First egg laid at 282 days; mother's first egg at 299 days.
31	♀	1.3	No egg laid at 307 days; mother's first egg at 299 days.
			Injections of fresh whole suprarenal
32	♂	3.1	Alive at 12.0 mo.; normal growth and behavior.
33	♂	1.8	Dead at 9.4 mo.; tuberculous; growth normal.
34	♀	2.7	Died of first injection.
35	♀	2.5	Killed 4 days after fourth injection; reproductive organs unaffected.
36	♀	1.2	First egg laid at 208 days; mother's first egg at 158 days.

TABLE 1.

Special data concerning young ring doves into which suprarenals from related doves were repeatedly transplanted.

Sex	No. of Bird	No of pairs suprarenals transplanted		Age (days) at beginning reproduction		Weight		
		Before maturity	After maturity	Treated bird	Mother of treated bird	Body grams	Suprarenals (mgms.)	Gonads (mgms.)
				TRANSPLANTED SERIES				
Female	1	1	2	160	251	163	10.4	
Female	2	1	2	136	147	162	9.5	
Female	3	2		169	267	143	8.0	153
Female	4	4		141	204	139	10.1	95
Female	5	4		155	173	169	13.2	295
Female	6	5	2	183	251	124	11.2	132
Female	7	5		153	239	157	9.2	
Female	Average			157	204	151	10.2	169
Male	8	1	1		190	152	8.7	902
Male	9	2			239	149	7.6	1108
Male	10	3			190	154	10.2	1013
Male	11	5			173	168	12.7	1257
Male	Average				198	156	9.8	1070
				CONTROL SERIES				
Female	12			213	357	162	10.7	
Female	13			180+*	380	179	13.1	
Female	14			183	153	161	9.7	
Female	15			199	181	144	6.3	
Female	16			132+*	239	132	11.0	262
Female	17			159	275	156	12.5	160
Female	18			146	215	170	8.6	
Female	19			131	235	168		
Female	20			165+	173	166	10.8	290
Female	Average				245	160	10.3	237
Male	21				215	149	9.7	691
Male	22				357	154	8.5	128
Male	23				156	161	12.2	886
Male	24				165	180	12.0	1117
Male	Average				223	161	10.6	706

* Killed at this age and before having produced an egg.

229 (2189)

The relation between the chylomicrons (free granules) and the lipoid content of the blood.

By ARTHUR KNUDSON and W. K. GRIGG.

[*From the Laboratory of Biological Chemistry, Union University Medical Department, Albany Medical College, Albany, New York.*]

When blood is examined under a dark field microscope numerous small brilliant dancing particles are visible which are invisible under the bright field microscope. These particles have been called by various investigators elementary particles or granules, free granules, blood dust, fat dust, hemaconia, and ultra or micro particles. Recently, Gage[1] and Gage and Fish[2,3] have made an extended study of these ultra particles or free granules and they have shown conclusively that the appearance of numerous free granules in the blood is dependent upon the fatty portion of the diet. Carbohydrate or protein diets in man cause no increase in the number of these particles in the blood but if fat is mixed with the diet or taken alone there is a marked increase under normal conditions. Since these granules appear in the blood after ingestion of fat Gage and Fish[2] believe that they must get into the blood by means of the chyle vessels and the thoracic duct and suggest the term chylomicrons (microscopic bodies from chyle) to designate these particles.

Gage and Fish[2] have devised a method by which these chylomicrons can be counted and estimations made as to their amount present in the blood. Although complete accuracy cannot be claimed in counting these particles, the counts are fairly accurate and the blood under various conditions can be conveniently studied. Since these chylomicrons have been shown to be dependent upon the fat absorbed from the diet and are therefore probably visible fat particles it was thought that the determination of the chylomicrons by the method of Gage and Fish[2] would give an index of the total amount of fat present. As the method is simple and requires only a small

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1 Gage, S. H., *Anat. Rec.*, 1920, xviii, 235.

2 Gage, S. H., and Fish, P. A., *Jour. Am. Vet. Med. Assn.*, 1921, lviii, 384.

3 Gage, S. H., and Fish, P. A., *Cornell Veterinarian*, 1921, xi, 143.

amount of blood it is obvious that it might be of distinct advantage in clinical work.

With this point in view several experiments were carried out determining the number of chylomicrons in the blood by the Gage and Fish method[2] and the total cholesterol and total fatty acids by the method of Bloor, Pelkan and Allen.[1]

The experiments were as follows:

Experiment 1—Dog 14 was a healthy female mongrel weighing 7.75 kg. A specimen of blood was taken and the dog then fed by mouth 70 c.c. of olive oil. Specimens of blood were then taken two hours after feeding and every two hours thereafter up to eight hours.

Experiment 2—Dog 15 was a healthy male weighing 17 kg. A specimen of blood was taken and then the dog was fed by mouth 70 c.c. of olive oil. Specimens of blood were taken as in Experiment 1.

Experiment 3—Dog 14 as above was given 25 c.c. of glycerol mixed with about 100 c.c. of water and specimens of blood taken before feeding and every two hours thereafter.

TABLE I.

Number of Experiment.	Time.	Whole Blood.		
		Total Cholesterol.	Total Fatty Acids.	Number of Chylomicrons.
1		mg. per 100 c.c.	mg. per 100 c.c.	
	Before	180	261	19
	2 hrs.	178	290	180
	4 hrs.	172	314	150
	6 hrs.	173	322	186
	8 hrs.	173 —	471	156
2	Before	171	281	8
	2 hrs.	172	323	145
	4 hrs.	180	299	121
	6 hrs.	184	307	99
	8 hrs.	180	423	170
3	Before	191	352	37
	2 hrs.	194	355	40
	4 hrs.	196	345	62
	6 hrs.	191	353	100
	8 hrs.	182	348	89
4	Before	68	156	51
	24 hrs.	66	209	37
	48 hrs.	122	870	73
	72 hrs.	198	1278	49
	96 hrs.	141	878	25

[1] Bloor, W. R., Pelkan, K. F., and Allen, D. M., *J. Biol. Chem.*, 1922, lii, 191.

Experiment 4—Rabbit 20 was a male weighing 1.60 kg. Lipemia was produced in this rabbit by extensive bleeding. First day 35 c.c. of blood was withdrawn, second day 25 c.c., third day 30 c.c., and fourth day 20 c.c. The diet was carrots and cabbage and remained the same throughout the experiment. Blood was taken each morning before feeding.

The analytical results are given in Table I and the curves below show the relation between the chylomicrons and total fatty acids more graphically.

EXP. 1   DOG
70 CC. OLIVE OIL

EXP. 2   DOG
70 CC. OLIVE OIL

EXP. 3   DOG
25 CC. GLYCEROL

EXP. 4   RABBIT
ACUTE HEMORRHAGE
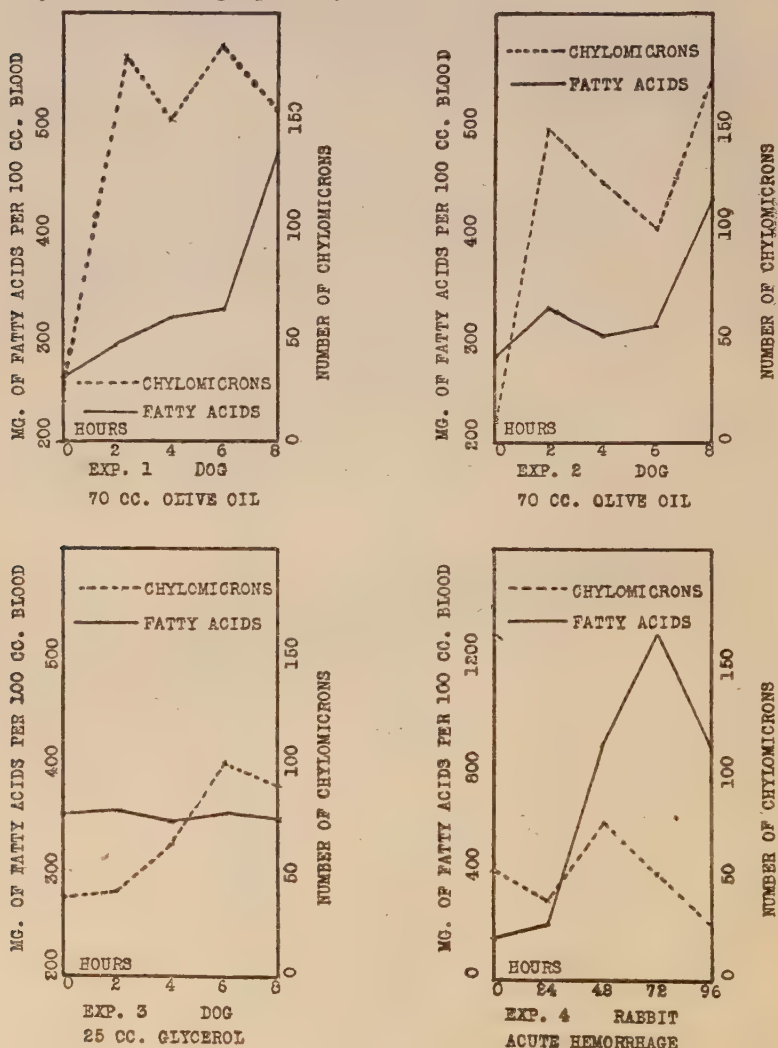
The table and curves show that there is no constant relation between the number of chylomicrons and the total fatty acids. In experiments 1 and 2 the chylomicrons reach their maximum in about two hours while the total fatty acids do not reach a maximum until eight hours. Usually the height of the curve for total fatty acids occurs in from 4 to 6 hours after a fat meal but in these two dogs there must have been delayed absorption.

In experiment 3 there is an increase of the chylomicrons after feeding glycerin but no appreciable change in the total fatty acids. This increase in chylomicrons is similar to experiments reported by Gage and Fish[2] who were inclined to the view that a sufficient amount of fatty acids can be furnished by the tissues to build up fat from glycerin that has been ingested. Other investigators have shown that if fatty acids alone are administered the tissues are able to furnish sufficient glycerin to form fat, and, if the chylomicrons are taken as an index of the amount of fat in the blood it would indicate that the reverse is possible. However, the total fatty acids remain constant throughout this experiment and the increase in chylomicrons is difficult to explain except on the basis that possibly some of the soluble fat in the blood is changed to a visible insoluble fat.

In experiment 4 the total fatty acids are increased out of all proportion to the increase in chylomicrons. As observed by Horiuchi[1] and Bloor[2] the lipemia produced in rabbits by acute hemorrhage shows a marked increase in cholesterol as well as total fatty acids. The hemorrhagic lipemia has been shown by Horiuchi[5] to be produced on a fat free diet and therefore the increased fat may then originate mainly, if not entirely, in the fat stores. If the chylomicrons are fat, then the fact that there is no great increase of these in this type of lipemia corresponding to the great increase of fatty acids would indicate that a large part of the fat is present in the blood in a form not visible under the dark field microscope.

CONCLUSIONS

These experiments indicate that there is no constant relation between the total fatty acids and the chylomicrons in the blood after feeding of fat and glycerin, and in the lipemia of rabbits

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1 Horiuchi, Y., *J. Biol. Chem.*, 1920, xliv, 363.
2 Bloor, W. R., *J. Biol. Chem.*, 1921, xlix, 201.

produced by acute hemorrhage. The determination of the chylomicrons, therefore, cannot be taken as an index of the total fat in the blood.

That the chylomicrons are associated with the fats in the blood is confirmed.

230 (2190)

A hitherto undescribed pair of isoagglutination elements in human beings.

By ARTHUR F. COCA and H. KLEIN.

[*From the Department of Bacteriology and Immunology, Cornell University Medical College and the New York Hospital, New York City.*]

Landsteiner, in 1901, discovered three human blood groups, and soon afterward, Decastello and Sturli, pursuing the study with Landsteiner's knowledge and encouragement, found the fourth group. Landsteiner recognized the existence in human serum of two isohemagglutinins, which were designated with small letters a and b, or (by von Dungern and Hirschfeld) a and β and two isoagglutinable substances in the corpuscles which were called A and B.

Quite recently Guthrie and Huck have reported the discovery of a third pair of isoagglutination elements, which, to bring the terminology into conformity with the previously existing one, we will refer to as isoagglutinin c and agglutinable substance C.

The relation of the exceptional individuals observed by Guthrie and Huck to the original four blood groups is shown in Table I.

TABLE I.

Groups	I	II	III	IV
Serum	a. b.	b	a	....
Corpuscles	....	A	B	A. B.
Guthrie and Huck		b A. C.	c B	

In the course of a class demonstration of the two original pairs of isoagglutination elements, the serum of one of us (A. F. C., a Group I individual) was absorbed, first with the washed corpuscles of a Group II individual (Levine) and then with the washed corpuscles of a Group III individual (Sutton). The supernatant fluid, after these absorptions, no longer clumped the corpuscles of the respective Group II and Group III individuals, but still possessed a vigorous power of agglutinating the corpuscles of a Group IV individual (Johnson) which were agglutinable by the serum of both Levine and Sutton.

This observation indicated the presence of another undescribed pair of isoagglutination elements, which we shall tentatively designate as x (isoagglutinin) and X (isoagglutinable substance).

Examination of 10 other Group I sera revealed the presence of "x" in 7 of them (see Table II).

It is evident that the agglutinable substance "X" is lacking in the Group II and Group III corpuscles that were used for the absorption. Six other Group III corpuscles have been examined and all were found to lack the agglutinable substance "X." Of 13 other Group II corpuscles only 2 have been found to lack this substance.

No other Group IV corpuscles have been available.

Six Group III sera have been examined and of these, 2 have been found to possess the isoagglutinin "x;" the other 4 lacked it.

TABLE II.

Group I Sera.		Unabsorbed tested with corpuscles of		After absorption with L and S corpuscles tested with corpuscles of		
		L	S	L	S	J
1	(A.F.C.)	+ +	+ +	0	0	+ +
2		+ + +	+ +	0	0	+ +
3		+ +	+ +	0	0	+ ∓
4		+ +	+ +	0	0	0
5		+ +	+ +	0	0	+ +
6		+	+	0	0	+ +
7		+ +	+ +	0	0	+ +
8		+ +	+ +	0	0	+ +
9		+ +	+ +	0	0	+ +
10		+ +	+ +	0	0	0
11		+ +	+ +	0	0	0

L = P. Levine (Group II lacking agglutinable X).
S = H. B. Sutton (Group III).
J = S. Johnson (Group IV).
+ = agglutination.

TABLE III.

Groups	I	II	III	IV
Serum	a. b. x.	b	a. c. x.	
Corpuscles		A. C. X.	B	A. B. X.

According to our present knowledge the constitution of the four blood groups, including the two new pairs of elements, may be represented as in Table III.

The possibility of this pair of isoagglutination elements being identical with that described by Guthrie and Huck must be considered. The study will be pursued and a detailed report will be made in the *Journal of Immunology*.

231 (2191)

A dangerous "universal donor" detected by the direct matching of bloods.

By PHILIP LEVINE and JENNIE MABEE (by invitation).

[*From the Department of Bacteriology and Immunology, Division of Immunology in Cornell University Medical College, and the New York Hospital.*]

In cases of transfusion where it is difficult to obtain a donor of the same group as the patient, a person of Group I (Jansky) has been considered suitable on account of the fact that the corpuscles of this group are inagglutinable by the isohemagglutinins. The fact that the plasma of a Group I individual contain isoagglutinins capable of clumping the corpuscles of the recipient has been ignored, because, under ordinary circumstances, the quantity of the plasma transfused is too small to affect the larger proportion (five to ten times) of the recipient's corpuscles.

In carrying out the method of direct matching of bloods described by Coca,[1] we found that the plasma of one of us (M., Group I) produced complete agglutination of ten volumes of the corpuscles of P. L. (Group II). Quantitative tests in the

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[1] *Ref. Journal of Immunology*, 1918, 93-100.

test tube showed that moderate agglutination of P. L. corpuscles was produced by as little as 1/20 volume of the M. plasma.

This observation reveals a risk in using a member of the so-called "Universal Donor" group for the first time without making a rough quantitative examination of the agglutinating power of the individual's plasma. It is advisable, therefore, in carrying out the direct matching according to Coca, to include a mixture of equal parts of undiluted recipient's citrated blood with the donor's citrated blood, diluted 1 to 5.

232 (2192)

Application of the murexide test to Amoeba verrucosa and Paramecium caudatum.

By RUTH B. HOWLAND (by invitation).

[*From Cornell University Medical School, New York City.*]

Observations made on the distended contractile vacuoles of Amoeba verrucosa subsequent to the application of the murexide test give no optical evidence of the presence of uric acid in the vacuolar fluid. This result is not in accord with the generally accepted conclusions of Griffiths[1] who reported the production of "prismatic crystals of murexide" on application of this test to Amoeba proteus, Vorticella and Paramecium. Mass cultures of Amoeba verrucosa, killed in 50 per cent. alcohol, show the contractile vacuoles fixed at partial or complete expansion in a large percentage of cases, but a critical examination of the fluid of the vacuoles has never revealed the presence of uric acid crystals precipitated there by this method. Dark field examination of such animals shows the fluid to be structureless. Treatment with nitric acid and ammonia in the manner described by Griffiths colors both cytoplasm and pellicle a lemon yellow. Furthermore, control slides, consisting of a small quantity of pure uric acid crystals in distilled water, when similarly treated, also give a negative result. Such controls give ammonium pur-

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1 A. B. Griffiths, *Proc. Soc. of Edinburgh*, 1888-89, xvi.

purate only on being evaporated to complete dryness, a procedure obviously so drastic as to render it inapplicable in the case of protozoan cells where it is essential to preserve the vacuole and its contents intact. Mass cultures so treated leave a lemon yellow residue.

Cultures of Paramecium, concentrated by brief centrifuging, and subjected to the same test also offer only negative results. Examination of the vacuolar fluid is precluded in these forms by the contraction of the vacuoles during the process of fixation.

This would indicate, therefore, either that uric acid is not an end product of the katabolic activity of these protozoa, or that the murexide test is not sufficiently sensitive to give a satisfactory optical reaction in this case. In view of the latter possibility, other and more specific methods for determining the presence of uric acid in minute quantities, are being applied at the present time.

The complete paper will appear in the *Journal of Experimental Zoology*.

233 (2193)

Studies on the contractile vacuoles of Amoeba verrucosa and Paramecium caudatum.

By RUTH B. HOWLAND (by invitation).

[*From Cornell University Medical School, New York City*]

The contractile vacuole of Amoeba verrucosa is formed by the union of a variable number of lesser globules. Subsequent to each contraction a new series of contributary globules may appear in the same location as the preceding vacuole, but this is not invariably the case. Random formation of new vacuoles is common, either at some distance from the original organelle, or close by. The walls of two functioning vacuoles may lie in contact for some time without confluence.

The wall of the vacuole is easily indented with a blunt microneedle. A sharp-pointed microneedle will induce artificial systole by perforation of the wall of the vacuole.

A large vacuole, or a group of contributary globules, freed into the water by ripping open a cell under slight pressure, retain their identity in this medium for some time. A quick upward thrust of a blunt needle against a large vacuole apparently causes its rupture into the surrounding endoplasm, for an increase in fluidity is observed in this area. This would imply the miscibility of vacuolar fluid and endoplasm.

The contractile vacuoles of Paramecium caudatum become dilated on the addition of Grubler's Alizarin blue to the culture. The distended vacuoles together with their feeders become set, gradually taking on a vivid blue color. This stain is specific for the vacuolar walls, and, although it appears long before ciliary action ceases, should be classified as a sub-mortem rather than a vital stain. These stained and distended vacuoles do not collapse when removed from the animal but retain their shape and may be manipulated and dissected with microneedles.

The complete paper will appear in the *Journal of Experimental Zoology*.

234 (2194)

Notes on the dissection of amoeba verrucosa.

By RUTH B. HOWLAND (by invitation).

[*From Cornell University Medical School, New York City.*]

The specific structure and characteristic properties of the pellicle of Amoeba verrucosa may be demonstrated in the living animals by means of microdissection and injection. A high degree of resistance to mechanical pressure is exhibited by the pellicle when subjected to compression. Perforation of the pellicle without indentation of this layer can be effected only with needles having extremely fine points. Withdrawal of a needle after penetration carries the pellicle out into a long cone-shaped process, loss of endoplasm being prevented by the rapid formation of a restricting endoplasmic film at the base of this cone. Distortion of the pellicle due to compression or other injury persists for some time before the membrane resumes its

normal condition. The tough but extensile nature of the pellicle may be further demonstrated by inflating the cell with distilled water injected through a micropipette. From a cell ruptured under pressure the entire pellicle may be removed. In every case recorded the nucleus came away with the pellicle. Injection of various solutions usually causes an immediate inflation of the Amoeba, the fluid collecting in an area beneath the pellicle, while the endoplasm contracts into an irregular mass containing the nucleus.

235 (2195)

The reaction of the endocrine system of the rabbit to tumor inoculation and the relation of this reaction to malignancy.

By WADE H. BROWN and LOUISE PEARCE.

[*From the Laboratories of the Rockefeller Institute for Medical Research, New York, N. Y.*]

Within recent years, a great deal of evidence has been accumulated from both clinical and laboratory sources which has tended to show that some kind of connection exists between the occurrence and growth of tumors and the system of organs regulating animal economy. For the most part, the evidence bearing upon this problem has been circumstantial. In a few instances a definite relationship appears to have been established as in the case of the experiments reported by Loeb[1] concerning the effects of castration on the incidence of mammary tumors in mice and those of Rohdenburg, Bullock, and Johnson[2] on the effects of operative removal of various organs upon the growth of transplanted tumors and the immunity of tumor animals. There are also therapeutic observations on the use of thyroid and of thymic products alone or combined with castration[3] which might be regarded as equally suggestive were it not for the fact that similar results have been obtained by the use of a variety of means.[4]

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[1] Loeb, L., *J. Med. Res.*, 1919, xxxv, 447.

[2] Rohdenburg, G. L., Bullock, F. D., and Johnson, P. F., in *Studies in Cancer (George Crocker Research Fund)*, 1913, iii, 87.

[3] Beatson, G. T., *Glasgow Med. J.*, 1913, n. s. lxxvi, 81, and earlier reports.

[4] A more recent article on this subject has been published by Enge in the *Ztschr. f. Krebsforsch.*, 1923, xix, 339.

Two years ago, we reported the occurrence of a malignant tumor in the scrotum of a rabbit infected with *Treponema pallidum*.[5] Since that time, a careful study has been made of the conditions presented by this animal[6] and a long series of investigations based upon the behavior of transplanted tumors derived from this stock has been carried out which have shown that there is an essential connection between the growth of transplanted tumors and certain members of the endocrine system on the one hand, and the mechanism of animal defense on the other.

In the animal with the spontaneous tumor, there was a notable tendency to atypical epithelial proliferation in many parts of the body associated with a deficiency in the reaction of the surrounding connective tissues and a widespread tendency to degeneration. There were also very striking alterations in such organs as the thyroid, the thymus, the suprarenals and the lymphoid tissues, which might have been regarded as part of a general organic deterioration attributable to one or both of the diseases present. From our knowledge of the biology of syphilitic infections and from what is known as to the influence of this system of organs on animal economy, however, we were led to suspect that the changes present might not be entirely incidental. This suspicion was strengthened when it was found that inoculation of other animals gave rise to decided alterations in a number of the organs of internal secretion as well as in the lymphoid tissues. With a view to determining whether these changes were consequences of disease or possessed a functional significance in tumor-bearing animals, especial attention was given to the alterations that occurred in this system of organs.

EXPERIMENTAL

Since the fall of 1921, the thyroid, the parathyroids, the suprarenals, the hypophysis, the pineal, and the thymus of all animals inoculated with this tumor have been studied grossly and histologically, including determinations of weight per unit of body weight. As a means of control, like observations were made upon normal rabbits derived from the same stocks and

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5 Brown, W. H., and Pearce, L., Proc. Soc. Biol. and Exper. Med., 1921, xviii, 201.

6 Brown, W. H., and Pearce, L., *J. Exper. Med.*, 1923, xxxvii, 601.

kept under the same conditions as the tumor animals. The sacrificing of these animals was so arranged as to provide control observations on the condition of the glands existing at the time inoculation was carried out as well as at the time of determining the effect of the inoculation. The results of tumor inoculations were further controlled by the examination of a large series of animals with various forms of spontaneous disease and of animals inoculated with *Treponema pallidum* as reported elsewhere.[7]

Some of the experimental animals died as a result of tumor growth while others were either arbitrarily killed at intervals of from 48 hours to 4 weeks after inoculation or at some critical period during the progress of the disease. The results thus obtained were analyzed with reference to the growth and the malignancy of the tumor as indicated by the clinical course of the disease and the conditions existing at the time of death. The control animals with other diseases were handled in much the same way.

<div align="center">RESULTS</div>

Stated briefly, these investigations showed conclusively that the growth of the tumor was associated with the occurrence of marked alterations in the size and the general appearance of such organs as the thyroid, the thymus, and the suprarenals and that less pronounced changes took place in the parathyroids and the hypophysis. Distinct histological changes also occurred in the thyroid, the thymus, and the hypophysis, but no definite change could be made out in the other glands.

In general the initial change was of the nature of a hyperplasia associated at first with a reduction in the weight of the gland which was followed by hypertrophy, or an increase in weight, while the ultimate condition presented depended upon the course of the disease. Thus, where the tumor progressed for a considerable period of time with the formation of metastases, there was a marked increase in the weight of such organs as the thyroid and the suprarenals while the thymus remained small or diminished in size. If the disease assumed a highly malignant character as indicated by the early appearance

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[7] Brown, W. H., and Pearce, L., Proc. Soc. Exper. Biol. and Med., 1923, xx, 476.

of widespread metastases, all of the organs enumerated above showed a sharp reduction in weight suggesting an exhaustion. This occurred before the animal exhibited any clinical signs of physical deterioration.

If, on the other hand, the disease terminated in recovery, the picture presented was quite different. The thyroid invariably showed a hypertrophy which persisted through the early stages of resolution; it then diminished in size but increased again as healing was completed. The suprarenals showed a similar series of changes except that the reduction in size and the secondary increase occurred earlier than the corresponding changes in the thyroid. In these animals, the thymus showed an increase in weight which was proportional to the extent of the growth at the time regression set in and this was a striking feature of the reaction seen in animals capable of controlling the growth of the tumor as compared with those in which the reaction was ineffectual.

Again, in recovered animals and in animals that had been subjected to repeated inoculations of tumor emulsions (immune animals), the weights of the thyroid, the parathyroids, the thymus, the suprarenals, and the hypophysis were all found to be greater than in normal controls and they apparently retained this increased size indefinitely.

Finally, a similar series of changes occurred in the superficial lymphnodes and in the spleen. The lymphnodes showed an initial hyperplasia and enlargement followed by a terminal atrophy in acutely fatal cases. The spleen, on the other hand, showed comparatively little change during the early stages of the disease but later became enlarged. It was a notable fact that the enlargement was usually proportional to the atrophy of the thymus except in cases of fulminating malignancy where both organs not infrequently showed a reduction in size.

The alterations described showed clearly that there was an intimate relation between the reaction that occurred in these organs and the malignancy displayed by the tumor or the ability of the animal to control or suppress its growth. Viewed from another standpoint, a similar relation was found to exist between the occurrence of periodic variations in malignancy and the prevailing state of the endocrine mechanism as indicated by the weights of the glands of normal rabbits. For example,

during the 2 years that the tumor has been under investigation, it has shown distinct seasonal variations in malignancy which have followed a definite order. During the summer, the disease has been more benign than in winter while each spring and fall there has been a marked increase in malignancy as determined by such criteria as the rate of growth of primary tumors, the incidence and distribution of metastases, the proportion of cases of fulminating malignancy and the mortality of inoculated animals.

These variations in malignancy, or in animal resistance, coincided with the existence of a given state of equilibrium or the occurrence of readjustments of endocrine balance as shown by the weights of these organs in control animals. But, the periods of greatest malignancy occurred at the time of readjustment from winter to summer or from summer to winter conditions, that is, at periods of unstable equilibrium.

From these observations, the general conclusion was reached that the malignancy displayed by this tumor was largely a function of animal economy and that the resistance of the animal to the growth of the tumor was conditioned upon the activity of those organs ordinarily concerned in the regulation of growth and maturity.

236 (2196)

Animal resistance and the endocrine system of the rabbit in experimental syphilis.

By WADE H. BROWN and LOUISE PEARCE.

[*From the Laboratories of the Rockefeller Institute for Medical Research, New York.*]

In the course of the work that has been carried out in this laboratory during the past 8 years, it has become more and more apparent that of the several factors concerned in determining the general course and severity of syphilitic infections, the spirochete is of minor importance as compared with a number of other influences, especially the factor of animal resistance.

This is strikingly illustrated by the experimental infection in the rabbit which, as a rule, is comparatively mild but in exceptional instances may assume a more malignant form or persist in an active state with severe constitutional symptoms for months or even years and in the end cause the death of the animal.

Little has been known as to the nature or source of the forces employed by the animal in combating this disease but there is a great deal of evidence to indicate that resistance to this infection is a function of animal economy and is subject to the same influences as are concerned in the regulation of growth and development, and the maintenance of general metabolic activities. Moreover, there have been specific indications that resistance was influenced to some extent by the endocrine system.

This possibility was first suggested to us by the occurrence of marked seasonal changes in the severity of the disease but was most forcibly impressed upon us by the abnormal resistance displayed at times by pregnant and lactating females. Finally, when we came to investigate the crossed immunity relationships between syphilis and a malignant tumor which had developed in a syphilitic rabbit,[1, 2] we were again impressed by the importance of the constitutional element in resistance. In the case of the tumor, it was perfectly obvious that we had to deal with the endocrine mechanism and this led us to carry out on rabbits infected with *Treponema pallidum* a parallel series of investigations intended in part as a control for the work on the tumor but primarily as a means of determining whether any definite changes which appeared to bear a relation to the resistance displayed by the animal to syphilitic infection could be detected in the animal organism.

EXPERIMENTAL

Since November, 1921, a total of 139 rabbits infected with virulent strains of *Treponema pallidum* has been killed and examined at various stages of the infection, the time ranging from 1 week after inoculation (first incubation period) to several months after the development of complete latency. All organs were weighed and studied grossly and microscopically and the conditions found were analyzed with reference to the

1 Brown, W. H., and Pearce, L., *J. Exper. Med.*, 1923, xxxvii, 601.
2 Pearce, L., and Brown, W. H., *ibid.*, 631.

function of animal resistance as indicated by the course of the disease and the conditions existing at the time the animal was killed.

The results obtained from these examinations were controlled by similar observations made on a larger series (182) of normal rabbits derived from the same sources and comparable as to age, sex, breeds, length of caging, diet, and time and mode of killing. As a further means of control, similar observations were carried out on 51 rabbits with various forms and degrees of acute and chronic infections of spontaneous origin and by a comparison with effects produced by tumor inoculation and by inoculating first with *Treponema pallidum* and then with the tumor or *vice versa*.

RESULTS

The results of these experiments cannot be reported in detail but they show conclusively that, apart from any localized lesions which might be attributed to syphilis, there were definite changes in the weights of such organs as the thyroid, the parathyroids, the suprarenals, the hypophysis and the thymus, as well as in the liver, the spleen, and the lymphoid tissues in general. The majority of these organs showed distinct changes in appearance and structure which were especially noticeable in the thyroid, the parathyroids, and the thymus. Moreover, the status of the organs at one stage of the infection differed from that at another and, in general, the direction and magnitude of the change that occurred was proportional to and parallel with the resistance displayed by different groups of animals. Finally, it was found that the changes which took place as the disease was brought under control and immunity was established were of a more or less permanent character. In other words, such organs as the thyroid, the parathyroids, the suprarenals, and the thymus, did not revert to their normal size and appearance but maintained a condition suggestive of a higher plane of functional activity and of a change in the general relationship of one organ to another.

In severe infections, some of these glands showed unmistakable evidences of injury. This was most noticeable in the case of the thyroid and the parathyroids but on the whole it appeared that the changes were related to alterations in functional activity which had to do with the development and maintenance of the resistance to infection.

Upon the basis of these findings, a further series of experiments was undertaken by Louise Pearce and C. M. Van Allen for the purpose of determining whether the course of the disease could be altered by operative interference with any of the endocrine glands or by the administration of such chemical agents as the iodides and mercury. Neither of these agents possesses any considerable spirocheticidal action in doses below the lethal limits but have been found to produce marked changes in the endocrine glands and the lymphoid tissues.

These experiments are not complete but they show already that, by either of the methods mentioned, the general character and course of the disease may be greatly modified; that the resistance of infected animals may be augmented or depressed by one and the same agency; that the immediate effect is proportional to the stimulation or depression of the activity of this system of organs. In other words, it has been found that the development and resolution of syphilitic lesions are subject to the same influences as are concerned in ordinary processes of growth and maturity. In this instance, it is of interest to note that the lymphoid tissues act in harmony with the endocrines or as though they formed a part of the general system of organs concerned in these reactions.

DISCUSSIONS AND CONCLUSIONS

The experiments referred to open up an enormous field for investigation. They supply us with a concrete basis for dealing with phenomena of disease which are dependent upon animal behavior. In fact, they point to the possible importance of this system of organs wherever the factor of constitutional predisposition or resistance enters into the problem of etiology or resistance to disease. At the same time, the fact cannot be emphasized too strongly that we are dealing here with a system of organs and tissues whose action may be interfered with at a number of points and in a number of ways which in the end may produce effects almost identical in character. In other words, while it is obvious that one or another of the elements of this system may be immediately responsible for a given effect, or of greater importance than another, the integrity and stability of the mechanism and the existing balance in the functional activity of its component parts appear to be the factors of foremost importance.

The deductions which might be drawn from the experiments already carried out are numerous. These experiments not only supply us with a better basis for understanding phenomena of constitutional resistance or susceptibility to syphilitic infection in general but they throw considerable light upon many obscure aspects of the disease. It is not unlikely that differences in individual susceptibility and differences between the sexes and between pregnant and non-pregnant women or even racial and geographical peculiarities of the disease may be explainable upon this basis. In the same way, other features of the disease, such as the abnormalities of detention in congenital syphilis, may be attributable to the injury inflicted upon this group of organs. Again, it appears that the maintenance of immunity is in some way dependent upon the functioning of this mechanism at a new and probably a higher level which is necessitated by the presence of an infection which must be kept under continuous control. This state of heightened activity or constant stress may in turn account for the eventual physical deterioration and predisposition to other forms of disease which so often occur in syphilitic individuals.

Finally, these experiments also serve to indicate what may be accomplished by a system of therapy that is intended to reinforce the resistance of the patient. At the same time, they serve as a warning of the dangers that may be encountered by attempting to treat syphilis without due regard for the effects of such treatment upon the patient or from ill advised efforts to alter the action of the mechanism concerned in maintaining constitutional resistance.

237 (2197)

Reflex contractions of an all-or-none character.

By EUGENE L. PORTER and VICTOR W. HART (by invitation).

[*From the Department of Physiology, School of Medicine, Western Reserve University, Cleveland, Ohio.*]

Sherrington's emphasis on the differences between reflex arc conduction and conduction in the nerve trunk has long been familiar to physiologists. With the establishment of the all-or-none law for muscle and for nerve in recent years it was but natural that the query should be raised as to whether reflex action be bound by the limitations of the all-or-none behavior of muscle and of nerve, or whether the central nervous system so alters the final nerve impulse to muscle that it can over-ride these limitations. A single sensory impulse, for example, might always result in a volley of impulses along the final motor neurones, according to Sherrington's suggestion.[1] Variations in the number of impulses comprising this volley might, by summation of contractions, conceivably produce in the muscle degrees of response of almost any grade of fineness, and reflex contractions would not then show an all-or-none character.

The present research was undertaken with the object of comparing minimal, or close to minimal, responses of muscle secured by single shocks to its motor nerve, with similar responses obtained reflexly by stimulation of a sensory nerve.

The muscle employed was the tenuissimus, a long slender, straight-fibered muscle underlying the biceps and containing approximately one thousand fibers.[2] The nerve to this muscle contains about twenty motor neurones, each neurone presumably innervating approximately fifty muscle fibers. If this nerve be stimulated by single shocks gradually increasing in strength the response of the muscle is not gradual, but by step-like increments, there being no gradation between one step and the next. The heights of the first three increments to appear in three of such experiments are shown in Experiments I, II and III of the appended table. The response is of the same character as that observed by Lucas[3] in the dorso-cutaneous nerve muscle preparation in the frog. When the appropriate sensory nerve is sim-

1 Sherrington, *Proc. Roy. Soc.*, 1921, xcii, B, 245.
2 Graham Brown, *Proc. Roy. Soc.*, 1914, lxxxvii, B, 132.
3 Lucas, *Jour. Phys.*, 1905, xxxiii, 125.

TABLE OF RESULTS.

REFLEX AND NERVE-MUSCLE CONTRACTION HEIGHTS NEAR THE THRESHOLD OF STIMULATION.

Tenuissimus muscle of the cat. Single induction shocks gradually increased in strength applied to the motor nerve ("Nerve-muscle") or to a sensory nerve for reflex contractions ("Reflex"). Cat made spinal by pithing the brain. The first three contraction heights only are listed. Magnification approximately ten times. Optical registration.

			Height of record on drum in mm.		
			1st step.	2nd step.	3d step.
I	Nerve-muscle	(2-22-22)	9	18	25
II	Nerve-muscle	(6-27-22)	7	28	42
III	Nerve-muscle	(7-10-22)	28	40	57
IV	Reflex	(7-5-22)	35	44	50
V	Reflex	(7-14-22)	15	35	46
VI	Reflex	(7-20-22)	7	12	22
	Nerve-muscle		6	10	21
VII	Reflex	(11-17-22)	3	7	14
	Nerve-muscle		4	14	....
VIII	Reflex	(11-28-22)	8	11	14
	Nerve-muscle		6	14	23
IX	Reflex	(12-1-22)	13	26	32
	Nerve-muscle		10	37	....
X	Reflex	(9-25-22)	8	24	30
	Nerve-muscle		13	35	....

ilarly stimulated and reflex contractions of the muscle obtained the records show the same step-like increments in contraction heights (Exps. IV and V).

The intervals between one contraction height and the next are of the same order of magnitude as those of the nerve-muscle preparation. Experiments VI to X are cases where the records have been obtained from the same muscle in the same animal. Exact duplication of records is not to be expected but the correspondence in height of increments is close,—notably so in Experiment VIII. Each step or increment follows the all-or-none law; if an increment appears at all it appears at its maximum. The records therefore furnish presumptive evidence that in the vicinity of the threshold, reflex contraction does not escape the limitations of the all-or-none conditions under which nerve and muscle fiber act. To this extent the results confirm those of Olmsted and Warner,[4] and are in opposition to those of Graham-Brown.[5]

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[4] Olmsted and Warner, *Amer. Jour. of Physiol.*, 1922, lxi, 228.
[5] Graham Brown, *loc. cit.*

238 (2198)

A study of the pulmonary circulation by the transillumination method.

By HARRY L. HALL (by invitation).

[*From the Department of Physiology, Western Reserve University Medical School, Cleveland, Ohio.*]

Method: The lungs of a pithed cat are aerated by Meltzer's method of intra-tracheal insufflation, temporarily interrupted once per minute. A constantly inflated lobe is carefully elevated from the open thorax and its margin fixed by two serrated clips. If an area near the margin is transilluminated by a suitable optical system the alveoli with their related blood vessels can be directly observed and studied by means of a horizontally arranged microscope magnifying 50 to 100 diameters.

Owing to the wall-thickness of the intra-lobular arteries and veins and their immediate branches, the circulating blood is only visible in the smaller vessels, *i. e.,* (a) in the pre-arterioles and arterioles passing between the alveoli, (b) in the capillary network formed on the surface of the alveoli and embracing the air cells and (c) in the smaller venules before they merge into the efferent intra-lobular veins.

Circulation in Vessels of Different Size: In the *pre-arterioles* the blood stream is often clearly visible. The cellular elements are in densely packed mass formation, the flow is pulsating with a rapid systolic forward movement and a diastolic decrease in velocity, amounting occasionally to a total stoppage of the flow in diastole. Expansion and relaxation of the walls is not observed. In the arterioles the stream is no longer pulsating, the individual blood cells are distinguishable and travel in ranks of two to six. In the capillaries, the blood corpuscles travel in single file, and in a continuous stream, but the rate of flow varies in different capillaries even in the network surrounding the same alveolus. In the smaller venules the stream is constant and a little more rapid than in the capillaries. Individual cells are distinguishable and eddies often occur at the junctions. In still larger venules and the smallest veins the stream is no longer constant, but a definite pulsation occurs, the onward flow tending to be reduced during systole and increased during diastole.

Although many patient observations have been made on a large number of cats no change in caliber either of the pre-arterioles, the arterioles, capillaries or venules has been seen, nor has the appearance or disappearance of patent capillaries been observed as long as the degree of lung inflation or heart rate remain unchanged. There is therefore no evidence in this research of an active change in the size of the smaller pulmonary vessels.

Effect of Stimulating the Peripheral Vagi Nerves after previous injection of, or painting of Cardiac base with Nicotine.
Upon stimulation of the peripheral vagi nerves in animals where no change in heart rate occurred, a curious effect is generally observed. With the focus unchanged and a constant lung inflation the vessels appear to pass out of focus, the alveolar air cells appear to bulge, to become more globular and to assume a more glistening appearance. On refocusing, the vessels appear to have sunk deeper into valleys between alveoli. The possibility that these changes may result from stimulation of bronchomotor nerves suggest itself.

This change in appearance during stimulation of the vagi nerve makes it difficult to be quite certain in regard to changes in the size of individual vessels but observation on 24 different experiments failed to yield any evidence that could be safely interpreted as indicating a change in caliber of any vessel, or an alteration of the blood flow therein. Evidence of vasomotor activity which it was hoped might be revealed by the use of this method could not be adduced.

Effect of Cardiac Slowing: When stimulation of the peripheral vagus nerves in un-nicotinized animals causes a marked cardiac slowing, an unanticipated effect on the blood flow in the visible pre-arterioles and arterioles is noted. During diastole the blood actually flows backward, out of the capillaries and smaller arterioles and toward the larger arteries; during systole, this is momentarily checked. This reverse flow continues for a few beats only, however, becoming less and less with each heart beat until the flow becomes stationary. Then if slowing is maintained a gradual onward movement during systole becomes reëstablished. This obviously indicates that the pressure in the pulmonary artery and its immediate branches is lower than in the smaller vessels until sufficient time has elapsed to produce a stabilized effect.

Effect of Epinephrin: In spite of a cardiac acceleration, produced by the injection of 1-3 c.c. of a 1:50,000 epinephrin solution, a definite decrease in the size of the visible pre-arterioles and arterioles is noted. At times this amounts to a complete cessation of flow in certain vessels. This supplies visible corroborative evidence that epinephrin effects the smaller pulmonary vessels.

239 (2199)

The respiratory exchange and blood sugar curves of normal and diabetic subjects after epinephrin and insulin.

By RICHARD S. LYMAN, ELIZABETH NICHOLLS and W. S. McCANN.

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This communication is based on a series of experiments conducted on 5 normal men and 8 diabetics. The method of determining the respiratory exchange was carried out by the open-circuit Tissot spirometer, with gas analyses by a modified Henderson-Haldane apparatus and calculations of the indirect calorimetry by the method of Zuntz and Schumburg, as recently described by McCann and Hannon.[1] At varying intervals during the experiments samples of blood were taken and blood-sugar determinations were made according to the method of Folin and Wu.[2] Coincident pulse and blood pressure charts were also kept in most instances.

The 5 normal men showed a similar reaction to 0.5 c.c. adrenalin given subcutaneously. There was a prompt rise of the R. Q., which was most marked about 10 minutes after the adrenalin. The quotient rose to a different extent, varying with the individual. A distinct increase of heat-production occurred with its maximum degree about 30 minutes after injection, remaining above the basal figure for over an hour. There appeared an immediate and marked rise in carbohydrate metabolism

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1 McCann and Hannon, Johns Hopkins Hospital Bull., March, 1923, xxiv, 73.

2 Folin and Wu, *Jour. Biol. Chem.*, 1920, xli, 367.

with its maximum about 10 minutes after the adrenalin, as calcu-
lated from the non-protein R. Q. Coincident alveolar air
analyses in one case indicated that overventilation may play a
rôle in the production of the higher respiratory quotients.
An elevation of blood-sugar followed the injection of adrenalin
reaching a maximum in 40-50 minutes after administration. It
was the most constant of any of the observed effects of the drug.
The sugar remained above normal limits for over $1\frac{1}{2}$ hours.
The pulse rate and blood pressure responded differently in dif-
ferent individuals.

The reaction to adrenalin of the diabetic patients differed
in some respects from that in the control cases. Its appearance
was the more delayed, the sicker the patient. The R. Q. did
not rise nearly as high as in the controls. There was only one
diabetic patient whose R. Q. went up as many points as the
minimum control. After adrenalin the eight diabetic subjects
fell into two groups as regards the total heat production :—four
showing an increase from 6 to 18 per cent., the rest ranging
from 29 to 43 per cent. above normal. The equivalent figures
for the five normal cases lay between 17 and 33 per cent. above
the basal heat production. The blood-sugar did not rise as high
nor as quickly as in the controls, with one exception. The
change was very slow in some cases. The blood-sugar fell in
one case, which was repeated with identical result.

The insulin reaction in normal subjects was observed in five
experiments in all, only two, however, having complete data.
Three and one-half units were usually given intravenously.
That dosage brought out a moderate reaction. Two and one-
half units caused a very slight reaction and five units required
the subsequent administration of sugar to relieve the symptoms
of hypoglycemia. The general course was similar in all experi-
ments of this group. There was a marked rise of R. Q., com-
ing to a maximum about 30 minutes after the injection of insulin
An increase of heat production occurred, reaching its highest
point later than the maximum R. Q., but returning to about
normal by two hours after the injection. There was a rapid fall
of blood-sugar which was at its lowest level from 20 to 30
minutes after insulin. By 40 minutes after a dose of $3\frac{1}{2}$ units the
blood-sugar was already beginning to rise, and by $2\frac{1}{2}$ hours
it had returned to about the normal figure. The diastolic blood
pressure usually fell. The systolic pressure rose 10 to 16 mm.

Hg. In one case in which sugar had to be later administered, the b.p. rose from 106/65 to 140/56. There was usually an in-increase of pulse rate (8 to 12 per min.), reaching its maximum 35 to 40 minutes after injection. This increase was never as mark-ed as when adrenalin was given to the same subject. Respiration was often slightly faster after insulin. The most marked sub-jective symptoms were twice noted to occur on the initial dose and in a similar repeated experiment the subjective sensations were minimal or absent, although the blood-sugar fell practically as low as on the initial injection. The subjective symptoms were of a different nature from those following the administration of adrenalin:—i. e., after insulin one noted weakness, chills or sweating, dimning of sense perceptions, mental haziness and wandering attention,—presumably due to the hypoglycemia.

In the experiments with diabetics the usual dose of insulin was ten units intravenously. The reaction was similar to that of the controls but the effects were slower to appear and less in extent, considering the dosage. The R. Q. invariably rose sooner or later after insulin. The maximum rise occurred later than in the case of any control at hand and it did not reach as high a figure as was obtained with normal subjects. In a num-ber of cases the R. Q. dropped slightly during the first half hour and then went up. This usually occurred on the initial dose of insulin but after a course of insulin therapy it later dis-appeared. Two of those subjects reacted but little to either adrenalin or insulin while two others showed marked response to adrenalin and relatively little to insulin. In the case of one normal subject the R. Q. taken ten minutes after insulin on one occasion and twenty minutes after on another showed a similar early drop of quotient. That subject was very sensitive to adrenalin. The extent of heat production varied. As a rule it did not increase markedly and it fell to or below the basal determination within two hours after insulin. The blood-sugar always fell. Its lowest level was reached considerably later than in the case of normal men and persisted at a low level consider-ably longer. In one patient the lowest level of blood-sugar moved nearer in point of time to the injection of insulin after a period of clinical improvement and the sugar curve also started to rise again sooner than it did on admission.

Intravenous insulin followed from 22 to 53 minutes later by

adrenalin subcutaneously showed a distinctly antagonistic rela-
tionship in the control cases.   In one case in which adrenalin
alone gave a marked rise of R. Q., practically the same reaction
to adrenalin resulted when it was preceded by insulin.   In an-
other case in which there was relatively little response to adren-
alin but more to insulin, the quotients followed the general di-
rection obtained when insulin alone was administered and there
was a fall of R. Q. on receiving the adrenalin after insulin.   The
calorigenetic action of adrenalin following insulin was always
less than that of the former when given alone.   The curves of
heat production after the combined insulin and adrenalin injec-
tions lay between the low one produced by insulin alone and the
higher one by adrenalin alone, and was closer to the one toward
which the subject reacted most when receiving the drugs sepa-
rately.   Adrenalin brought out distinct elevations of blood-
sugar but the rise was never as high when given after insulin as
when given by itself.   The pulse rate and blood pressure curves
gave the same general picture with the combination as with
adrenaline alone but the extent of circulatory changes was also
considerably less.

The effect of adrenalin following insulin in diabetics appeared
to depend upon the individual sensitiveness to the drugs and also
upon the condition of the patient,—e. g., severity of the sick-
ness, presumable glygogen stores, etc.   The R. Q.'s were in
agreement with blood-sugar changes. With two patients previous-
ly injected with insulin the quotient rose promptly after adrena-
lin; in two cases it fell.   The heat production in this group paral-
leled the curve of R. Q.   In two cases adrenalin after insulin made
the blood-sugar rise but in two cases the blood-sugar kept on
falling.   The two cases in which adrenalin failed to raise the
blood-sugar showed relatively little coincident circulatory re-
sponse to that drug and those patients had also previously proved
to be relatively insensitive to adrenalin when given alone.

240 (2200)

Method of titrating antigen for Kahn precipitation test.

By R. L. KAHN.

[*From the Bureau of Laboratories, Michigan Department of Health, Lansing, Mich.*]

This method aims to overcome variations in antigens employed in the precipitation test for syphilis proposed by the author. Two antigens prepared under identical conditions from two different lots of beef heart, will be likely to show variation in sensitiveness when tested with syphilitic sera. This variation in sensitiveness may be considerably lessened if each antigen is first mixed with salt solution in such proportion as to bring forth its maximum power for producing precipitates with syphilitic sera. Generally speaking, this is accomplished by mixing antigen with *minimum* amounts of salt solution which will produce opalescent mixtures. Since the mode of adding salt solution to antigen markedly affects the final product, it is obviously important to render all conditions which are likely to affect this titration as constant as possible.

The test tubes used in this titration should not differ from those used in the regular tests. In our experience, tubes of 0.8 to 1 cm. diameter give best results. The set up of the titration is as follows:

Six test tubes receive 0.5 c.c. antigen each. Six similar tubes receive respectively 0.25, 0.5, 0.75, 1, 1.25 and 1.5 c.c. salt solution (0.85 per cent NaCl). The 0.25 c.c. salt solution is now poured into the first antigen tube and the mixture immediately poured back and forth several times. The next antigen tube receives the 0.5 c.c. salt solution in a similar manner. The remaining antigen tubes receive the four increasing amounts of salt solution under similar conditions. It will soon be observed that the first two or three tubes show varying degrees of precipitation, the middle tubes, clouding and the last few tubes, opalescence.

The salt solution-antigen mixture in which the smallest amount of salt solution is capable of producing opalescence is the salt solution-antigen titre for procedure I.[1]

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1 PROC. SOC. EXP. BIOL. AND MED., 1923, xx, 325.

With most antigens, three parts of salt solution represent the minimum amount which will bring about an opalescent mixture. In some cases, however, 2.5 and even 2 parts of salt solution may still produce an opalescent mixture and may therefore be used with safety. An antigen-salt solution mixture must show no signs of turbidity when used in the tests.

Those tubes showing precipitation are now centrifuged for about 10 minutes, supernatant fluid poured off and replaced with an amount of salt solution (1 c.c.) equivalent to twice the amount of antigen originally used. After thorough shaking, it will be observed that whereas the tube which originally contained 0.5 c.c. antigen and 0.25 salt solution shows a precipitate, the next tube which contained equal quantities of antigen and salt solution, although milky, may be opalescent and entirely free from any suggestion of a precipitate. If this tube shows some turbidity instead of opalescence then the next tube which contained 0.5 c.c. antigen and 0.7 c.c. salt solution will be found to be opalescent. Under such conditions one is also likely to find that an antigen-salt solution proportion of 1:1.25 (instead of 1:1.5) will also produce opalescent mixtures and could therefore be used in the tests.

The salt solution-antigen proportion in which the smallest amount of salt solution produces a precipitate capable of forming an opalescent mixture on resuspension in salt solution is the antigen-salt solution titre in procedure II.

Table I illustrates a typical titration of antigen.

TABLE I.   ANTIGEN TITRATION.

Tube	1	2	3	4	5	6
Antigen c.c	0.5	0.5	0.5	0.5	0.5	0.5
Salt Solution c.c.....	0.25	0.5	0.75	1.0	1.25	1.5

The antigen and salt solution are mixed in each case by pouring the latter into the antigen tube and immediately pouring the mixture back and forth several times.

Results	Precipitate	Precipitate	Precipitate	Cloudy	Opalescence	Opalescence

Tubes 1, 2 and 3 are now centrifuged for about 10 minutes, supernatant fluid poured off, replaced with 1 c.c. salt solution (twice the amount of antigen originally employed) and mixed thoroughly.

Results	Precipitate	Opalescence	Opalescence			

This titration shows that in Procedure I two and a half parts of salt solution added to one part of antigen represent approximately the smallest amount of salt solution which will produce an opalescent mixture. The antigen-salt solution titre of this particular antigen, therefore, is

<div align="center">Antigen: Salt Solution = 1 :2.5</div>

In Procedure II, on the other hand, one part of salt solution added to one part of antigen appears to represent approximately the smallest amount of salt solution which produces a precipitate capable, after being freed from supernatant fluid, of forming an opalescent suspension in salt solution. The titre, therefore, is

<div align="center">Antigen: Salt Solution = 1 :1</div>

It is evident that the antigen titration outlined gives only approximate amounts of salt solution which produce opalescent mixtures. Occasionally one will find on trial that these amounts of salt solution can be slightly lessened and still obtain opalescent mixtures. Under these conditions, the smaller amount of salt solution should be employed in the tests.

After determining the smallest amounts of salt solution to mix with antigen in Procedures I and II, the next step is to test the opalescent mixtures with at least six positive and six negative sera, and particularly establish that there is no tendency for weak non-specific reactions.

One will not encounter non-specific reactions when employing opalescent mixtures. One should not confuse occasional sediments or precipitates settled on the bottom of the tube for specific precipitates which are almost invariably suspended in the medium. The employment of serum controls (0.3 c.c. serum + 0.05 c.c. salt solution) will overcome the uncertainty regarding

TABLE II.   RESULTS OF ANTIGEN TITRATIONS

	PROCEDURE I	PROCEDURE II
	Proportion of antigen and salt solution producing opalescent mixtures	
	Antigen: Salt Sol'n.	Antigen: Salt Sol'n.
Non-Cholesterinized antigen	1 : 1.75	1 : 0.85
Same antigen with 0.4 per cent. cholesterin..	1 : 2.5	1 : 1.1
Same antigen with 0.8 per cent. cholesterin..	1 : 3.5	1 : 1.4

these bottom precipitates, as they will be found to be present in the controls as well.

It may be of interest in this connection to give the titration results of an extract antigen prepared as outlined in a previous paper of these Proceedings,[1] and the same extract containing 400 and 800 mgm. of cholesterin per 100 c.c., respectively.

This titration would indicate that cholesterin plays an important rôle in necessitating proportionally larger amounts of salt solution to bring about opalescent antigen-salt solution mixtures. It might be added also that in a general way, cholesterin proportionally increases the sensitiveness of the antigen after mixing with syphilitic serum. The problems involved in the cholesterinization of antigen are reserved for further studies.

241 (2201)

Employment of different antigens in Kahn precipitation test.

By R. L. KAHN and W. W. DUEMLING.

[*From Bureau of Laboratories, Michigan Department of Health, Lansing, Mich.*]

Employing the antigen titration outlined in the previous paper, the question arose to what extent the preparation of antigen may be varied without affecting the final results. The following antigens were accordingly prepared, titrated with salt solution and smallest amounts of the latter used which produced opalescent antigen-salt solution mixtures. The tests were carried out with positive and negative sera according to procedures I and II.

Antigen 1. This was prepared as described in a previous paper of these *Proceedings*.[1] Dried beef heart was freed from ether extractives and subsequently extracted in 95 per cent. alcohol for 9 days in the ice box and overnight at incubator temperature. Color approximated potassium bichromate color standard.

Antigen 2. After extracting the dried heart with ether in the usual manner, boiling alcohol was poured on the dried material, shaken and extraction continued for 1 day in the incubator. Color approximated potassium bichromate standard.

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[1] Kahn, R. L., PROC. SOC. EXP. BIOL. AND MED., 1923, xx, 325.

Antigen 3. Same as antigen 2, except that the alcohol extraction was carried out in the water bath at 37.5° C. for 2 days with frequent shaking. Same color index as above.

Antigen 4. Same as Antigen 1, to which was added a second alcoholic extract carried out for 2 days at incubator temperature having approximately the same color range.

Antigen 5. Ether extractives were removed in the usual manner and dried heart muscle extracted with alcohol for three hours in reflex condenser.

Antigen 6. Ether extraction carried out for 1 hour in reflex condenser, followed, after filtration and washing with fresh ether and drying, by alcohol extraction also by means of a reflex condenser for 1 hour.

Antigen 7. Dried heart muscle was extracted in 95 per cent. alcohol for three days in the incubator. The alcohol was then filtered off, evaporated to dryness and taken up in a small amount of ether. About ten times the amount of acetone was then added, permitted to stay over night, the acetone decanted and the precipitate redissolved in absolute alcohol. (Noguchi.)

Antigen 8. Ether extractives were removed in the usual manner, and dried beef heart was extracted with alcohol for 3 days in the incubator. After filtration, the alcohol was evaporated to dryness, taken up in a small amount of ether and precipitated with an excess of acetone. The precipitate was finally taken up in absolute alcohol.

Antigen 9. Ether extract of *Antigen* 8 was evaporated to small volume, precipitated with excess of acetone and precipitate suspended in alcohol and placed in incubator overnight, filtered next morning.

Antigen 10. Equal quantities of antigens 8 and 9.

Antigen 11. Alcoholic solution of acetone insoluble product from ether extract of beef heart obtained after 1 hour extraction in reflex condenser.

Antigen 12. Equal quantities of Antigens 11 and 6.

Antigen 13. Ether extraction of dried beef heart was prepared and saved. An alcoholic extract was then obtained, evaporated to dryness and taken up in a small amount of ether. This was mixed with original ether extract and acetone insoluble antigen in alcohol prepared from it. This was finally mixed with a second alcoholic extract of the same dried muscle. (Kolmer.)

Antigen 14. Alcoholic solution of acetone insoluble product obtained from an ether extract of beef heart mixed with an alcoholic extract of the same beef heart obtained as outlined in Antigen 3.

To each of the above extracts were added 400 mgm. of cholesterin per 100 c.c. before testing with the various sera.

The results of preliminary experiments indicate that most of these antigens compare favorably with one another. In a general way the acetone insoluble antigens are somewhat weaker than the others. Necessarily, a large number of tests will have to be carried out before establishing the degree of sensitiveness and particularly the specificity of these various antigens.

It is of interest to note that "Antigen 6" which can be prepared in about three hours, appears to give unusually sensitive as well as specific reactions. This as well as the other antigens outlined are still being investigated and other antigens are under preparation.

242 (2202)

The elaboration and release of the colloid of the thyroid.

By EDWARD UHLENHUTH.

[From the Laboratories of the Rockefeller Institute for Medical Research, New York City.]

The development of the thyroid of the salamander *Ambystoma opacum* was studied on serial sections of thyroids in various stages before and after metamorphosis.

The proportion of colloid and epithelium was found by weighing separately wax models of the colloid and epithelium. During the larval period the colloid increases more rapidly than does the epithelium. From 13 per cent., shortly after hatching, it increases to 45 per cent. of the total thyroid mass just before metamorphosis. The larval period is not a period of colloid release, but of colloid elaboration and storage. At the beginning of metamorphosis, the colloid percentage drops suddenly below 30 per cent. This drop is due partly to the sudden and excessive release and disappearance of the colloid from the follicles, and partly to an excessive increase of the epithelial mass, owing to the swelling of the individual

cells. With increasing age the thyroid again enters upon a stage of colloid accumulation. In animals 4.5 years of age, 56 per cent. of the thyroid mass is colloid. This relative increase is not due entirely to an absolute colloid increase, but also to an absolute decrease of the epithelium.

The colloid was studied on sections stained, according to Kraus' method, with polychrome methylene blue and acid fuchsin.

Colloid is elaborated long before follicles are formed, at a time when the cells still contain yolk and are devoid of granules, a structure frequently considered to be prerequisite to colloid elaboration. In thyroids of larvæ fed exclusively thymus gland, the cells remain permanently primitive, consisting almost entirely of nucleus, possess very small amounts of plasma and are devoid of granules. Yet these cells elaborate large quantities of colloid (55 per cent. of the total thyroid mass).

At first colloid is elaborated only by the formation of intracellular colloid globules of small size which stain red, the color of newly formed colloid. Owing to the increase in the number of cells containing intracellular globules, two or more globules may touch each other, become free, and form, by fusion, a primary follicle. Or the globule may grow within the cell, become free by retraction of the surrounding cell plasma and form a primary follicle. Primary follicles form, by fusion, the large secondary follicles. Colloid elaboration by intracellular globules, now in the individual cells of the follicle walls and in the cells of the reserve cell masses, continues, even after the formation of secondary follicles. These globules may grow within the cell to a large size, become an extracellular colloid mass in the follicular wall, by retraction of the cell plasma, and fuse with the main colloid mass; or they may be extruded by the inner cell end directly into the follicle recognizable for some time by the red coloration. The latter is a permanent mode of colloid elaboration.

Another mode of colloid elaboration is the elaboration of colloid from granules, the predominant mode after secondary follicles have formed. At first, fine granules of reddish color are scattered throughout the plasma. Gradually they increase in number and in size and tend to crowd near the inner surface of the cell, where a reddish substance accumulates between the granules, apparently the result of the fusion of the granules. It seems that this substance can diffuse directly through the cell

membrane into the follicle, giving rise there to the marginal zone of red colloid around the yellowish brown, green, or blue center of the colloid mass.

Only one kind of stainable colloid is formed, the red colloid. It stains yellow, green, and finally blue, when it becomes old. It undergoes this change no matter if it ages in the follicle or within the cell. Blue colloid is not indicative of an actively releasing state of the thyroid, but of an accumulation of old colloid. Therefore, the colloid in the thyroid of old axolotls, which elaborate but do not release colloid, stains deep blue, almost black.

No explanation has been found as to how the colloid escapes from the follicle in the blood. It appears, however, that the vacuoles are somehow connected with the colloid release. There are two kinds of vacuoles in the colloid: closed vacuoles, and those which are in open communication with the inner cell ends. When the colloid first appears, it has no vacuoles. The colloid of all larvæ prevented from metamorphosing, by experimental procedures, and the colloid of larval axolotls are practically devoid of vacuoles. Apparently, the elaboration of stained colloid may take place in the absence of vacuoles. Yet, in the colloid of normal larvæ, the vacuoles increase steadily in number and size. Not only within the follicle, but also within the cell, the colloid develops vacuoles upon aging.

Communicating vacuoles are found only in thyroids releasing the colloid into the blood. They appear suddenly, when metamorphosis begins. At the same time, large vacuole-like spaces appear in the cells causing the swelling of the cells. It seems as if the content of the vacuoles is poured into the cells and escapes through the periphery into the blood. At any rate, communicating vacuoles are indicative of colloid release.

Feeding inorganic iodine to normal larvæ and axolotls, although it does not enforce the release of the colloid, produces ordinary vacuoles.

The elaboration of colloid is not necessarily followed by the release of the colloid. Feeding of inorganic iodine to old axolotl larvæ results in an increased rate of colloid elaboration, producing a wide marginal zone of red colloid; yet metamorphosis cannot be enforced by this procedure. Larvæ fed exclusively thymus, do elaborate colloid; yet they cannot metamorphose. Colloid elaboration and colloid release are, within certain limits, independent of each other.

243 (2203)

The mechanism of edema production by paraphenylenediamin.

By M. L. TAINTER and P. J. HANZLIK.

[From the Department of Pharmacology, School of Medicine, Stanford University, San Francisco.]

The subcutaneous injection of paraphenylenediamin hydro-chlorid in the dosage of 0.19 gm. per kilo in rabbits, produces a peculiar, specific edema of the head and neck in from one to three (median, one and one-half) hours after the administration. At the same time there is a relative increase in hemoglobin and total solids of the blood due to escape of fluid from the circulation, indicating that increase in vascular permeability may be a factor in the production of the edema. With this dosage, and providing the freshly dissolved drug is injected, edema is produced almost invariably. Old and standing solutions (even for 24 hours) are uncertain and ineffective, and this, in part at least, explains the variabilities previously encountered. The following is a summary of results on 96 animals which have been used for the study, in various ways, to date.

Meissner claimed that large doses of atropin and calcium prevented the edema. We have not been able to confirm this. Maximal doses of the following agents injected in various ways did not influence the development and the course of the edema nor the blood concentration; calcium, atropin, morphin, chloral hydrate, urethan, ether, sodium bromide, cocain, ergotoxin, antipyrin, neocinchophen, sodium salicylate, quinin and cinchophen (in some rabbits). In about half of the rabbits receiving cinchophen, the production of pleural and peritoneal exudates was favored and the mortality increased. Our results with atropin and calcium agree with negative results of Gibbs who used cats instead of rabbits for studying the edema of paraphenylenediamin. The only agent which has prevented the edema thus far is nicotin in large doses hypodermically. Section and

degeneration of the cervical sympathetic nerves did not prevent the edema.

Localization of the edema in the head and neck is not connected with an increased concentration of paraphenylenediamin in these regions, since quantitative estimations of the paraphenylenediamin in the saliva and edematous fluid showed the concentration to be less or no greater than that in the blood plasma.

The results with various concentrations of paraphenylenediamin (base and acid salt) on swelling of gelatin in aqueous solutions and of muscle in serum *in vitro* were negative indicating that the edema is not the result of change in the physical state of the tissue colloids by the paraphenylenediamin directly.

The edematous fluids in two rabbits gave P_H values of 6.86 and 6.95, while those of the blood were 7.1 and 7.2 respectively. In another animal, which received nicotin, the P_H of the edematous fluid was the same as that of the blood (*i. e.,* 7.3).

Paraphenylenediamin hydrochlorid oxidized by lead peroxide failed to produce edema. Hence, it appears that the oxidation products (quinondiimin, etc.) are not concerned in the edema.

The study is being continued.

244 (2204)

Increased number and clumping of thrombocytes (platelets) in pigeons produced by agents causing anaphylactoid reactions.

By F. DE EDS and H. A. SOMERFIELD (by invitation).

[*From the Department of Pharmacology, School of Medicine, Stanford University, San Francisco.*]

A variety of agents, previously reported by Hanzlik and Karsner cause anaphylactoid reactions when injected intravenously. With many of these emboli and thrombi composed of red blood corpuscles, fibrin and platelets are demonstrable in the lungs, and hemagglutination occurs *in vitro*. These changes together with the alterations in chemical composition of the blood recently demonstrated in this laboratory, are regarded as objective evidences of disturbances in important physical and chemical equilibria in the fluids and tissues of the organism, and as being of fundamental importance in the explanation of reactions from a variety of agents. The present report is a sum-

mary of the effects of different agents upon the thrombocytes (platelets) in pigeons.

For differentiation, the modified Nocht stain described by Hastings[1] was used and for counting, a modification of the cresyl violet stain of Buckman and Hallisey.[2] All agents were injected intravenously at body temperature into the wing veins and blood was obtained from superficial veins of the legs.

The following agents, which cause anaphylactoid symptoms in guinea pigs, pulmonary emboli and thrombi, and hemagglutination *in vitro*, produced increases in number and clumping of thrombocytes in pigeons; peptone, agar-sol gel, toxified agar, Congo red, collargol, charcoal, kaolin, colloidal iron, colloidal arsenic, 50 per. cent. acetic acid and 6 per cent. acacia. Histamin, tannin and arsphenamin (in small dosage) produced doubtful or no changes in the thrombocytes, but sections of lungs and livers showed marked clumping of erythrocytes from these agents.

Histological examinations of the lungs, liver, spleen and kidneys of all animals showed congestion and thrombosis after the majority of the agents that were injected. In a few cases marked hemorrhages were found. The majority of these agents caused definite symptoms, ranging from shivering, crouching, and increase in respiration to death.

On the other hand, the withdrawal of blood alone, and the injection of 0.85 per cent. sodium chloride (as control) produced no symptoms and no demonstrable changes in the thrombocytes; and histologically, the changes were slight or absent.

245 (2205)

The inorganic constituents of human saliva.

By GUY W. CLARK and G. S. SHELL.

[*From the Department of Biochemistry and Pharmacology, University of California, Berkeley, Calif.*]

There is a growing opinion that many of the pathological conditions of the oral cavity (caries, pyorrhea, etc.) are the result of faulty diets. The acceptance of this assumption makes it nec-

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1 Johns Hopkins Hospital Bull., 1904, 122.

2 *J. Am. Med. Assoc.*, 1921, lxxvi, 427.

essary to know the normal constituents of the saliva. During the last year several investigators have reported on one or more of the salivary constituents but a complete correlation between the dietary and salivary constituents is lacking. The work presented here is very incomplete and represents only one phase of a complete survey of the mineral metabolism being made on a number of adult human subjects.

The results thus far, given in a brief form in the following table, have been obtained from weekly analysis of saliva samples from six healthy adults, all of whom have been on a fixed diet for a periods of eight weeks.

SUBJECTS

	2	3	4	5	6	7
Total Solids—grams per 100 c.c.						
Min.	0.464	0.483	0.645	0.527	0.434	0.730
Max.	1.040	0.727	1.370	0.650	0.640	1.308
Avg.	0.752	0.576	0.865	0.585	0.537	0.923
Ash—grams per 100 c.c.						
Min.	0.176	0.127	0.175	0.148	0.123	0.194
Max.	0.274	0.220	0.371	0.250	0.220	0.292
Avg.	0.236	0.193	0.257	0.201	0.189	0.239
Cl—milligrams per 100 c.c.						
Min.	35.0	40.0	50.0	60.0	30.0	40.0
Max.	70.0	75.0	80.0	70.0	60.0	110.0
Avg.	53.0	58.0	66.0	62.0	40.0	66.0
P*—milligrams per 100 c.c.						
Min.	4.0	4.5	4.1	4.1	7.9	11.1
Max.	14.2	10.0	18.2	12.7	21.0	19.4
Avg.	11.2	8.4	12.8	9.4	12.6	15.3
N as NH_3—milligrams per 100 c.c.						
Min.	8.6	4.2	7.2	5.6	4.8	8.3
Max.	15.0	8.6	9.5	18.1	10.9	22.0
Avg.	10.6	6.0	8.2	11.5	8.9	14.8
Total N—milligrams per 100 c.c.						
Min.	54.2	44.4	60.0	45.2	51.0	82.7
Max.	69.1	74.1	72.5	70.0	56.9	99.0
Avg.	63.9	56.8	68.8	57.6	52.4	90.0
CO_2—bound as bicarbonate—c.c. per 100 c.c.						
Min.	8.7	8.7	4.9	3.0	13.4	10.6
Max.	23.9	14.3	26.7	9.6	17.2	15.3
Avg.	14.5	11.0	10.3	5.9	15.2	13.0
Ca—milligrams per 100 c.c.						
Avg.	6.9	......	5.6	......	5.3	......

* Acid soluble.

Further work is in progress.

246 (2206)

The aerobic cultivation of bacillus histolyticus.

By IVAN C. HALL.

[*From the Department of Bacteriology and Experimental Pathology, University of California, Berkeley, Calif.*]

A strain of *B. histolyticus* (No. 141) received from the Pasteur Institute in Paris in March, 1921, and described by me[1] last year, was recently found capable of repeated and successive aerobic culture upon the surface of meat infusion and blood agar slants without resort to any method of reducing the oxygen pressure. The accuracy of this observation is guaranteed not alone by the unique pathogenicity, but as well by the particular combination of morphologic and cultural properties of this species which enable one to identify it without reference to its oxygen requirements.

All authors dealing with *B. histolyticus* have regarded it as an obligate anaerobe and my own previous failure to observe aerobic growth can only be explained by my reliance upon meat extract, instead of meat infusion, agar slants, for detecting aerobic growth until recently. Even the growth upon meat infusion agar is extremely delicate and might be easily overlooked by any but a highly critical dye. My first supposition upon observing the delicate transparent aerobic growth upon a meat infusion blood agar slant was that a contamination had occurred. This assumption was clearly denied when as many as 31 successive transplants of this culture were made upon blood and plain meat infusion agar during a period of about 60 days without altering any of its morphologic, cultural or pathogenic characteristics permanently; there were temporary differences observed, however, in the morphology of the first few aerobic cultures, but later transplants appeared to be identical in every way with a corresponding strain cultured anaerobically in brain medium.

The possibility of contamination was also excluded by finding no significant differences in well separated deep agar colonies and in the observation that subcultures from such deep

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1 Hall, *Jour. Inf. Dis.*, 1922, xxx, 445.

colonies may also be grown aerobically. Deep agar colonies are distinctly larger, however, than those nearer the sufarce; there is usually, but not always indeed, a distinct zone of inhibition at and below the surface as with obligate anaerobes which is hard to explain if this species is a facultative aerobe-anaerobe, as my findings suggest.

That the above observations, limited at first to a single strain, were not to be interpreted as indicating acclimation of this strain to aerobic life, was shown when three other French strains received from Dr. Morton C. Kahn of Cornell University Medical School were also cultured aerobically. A fourth strain labelled *B. histolyticus* by Major Jablons and received from Dr. Kahn fail to grow aerobically and also failed to produce lesions in guinea pigs. But a strain of *B. histolyticus* recently isolated by my student, Miss Emelia Peterson, from a specimen of California soil grows both aerobically and anaerobically and has been transplanted aerobically three times with no apparent change in morphology, cultural characteristics or virulence.

<center>247 (2207)</center>

The isolation of bacillus histolyticus from soil in California.

By EMELIA PETERSON and IVAN C. HALL.

[*From the Department of Bacteriology and Experimental Pathology, University of California, Berkeley, California.*]

During a survey of California soils for anaerobic bacteria, we encountered a strain of *B. histolyticus* which is of interest as the first recovery of this species in America and one of the few records of its occurrence in soil. All of the other cultures so far described came from war wounds in France and the only recorded proof of this organism as an inhabitant of soil, aside from the fact that most war wounds are contaminated by dirt, is a statement by the Medical Research Committee[1] that, "it . . . has been obtained from earth."

The soil specimen was a clay adobe from near Walnut Creek, California, which lies in a rich agricultural valley of the coast range about 12 miles from Berkeley.

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*British Medical Research Committee, Report No. 39, 1919.

The filtrate of an initial culture of this soil in a meat mash medium in the constricted tube[1] contained also the toxin of B. botulinus Type A, and it was during our effort to recover this organism that the *Bacillus histolyticus* was isolated.

The primary culture contained numerous obligately aerobic hay bacilli and it is interesting to note that while our usual use[2] of gentian violet easily eliminated these by selective bacteriostasis, it was impossible in six trials to eliminate a certain facultative aerobe-anaerobe which we now consider to have been none other than the *B. histolyticus* since that was the only organism that could be isolated from the subsequent deep agar colonies.

The isolated culture corresponds in all of its morphologic cultural, and pathogenic properties to the war wound strains received from Dr. Weinberg of the Pasteur Institute of Paris or indirectly from Dr. Kahn of Cornell University Medical School.

248 (2208)

The failure of fermentation reactions with bacillus histolyticus.

By IVAN C. HALL.

[*From the Department of Bacteriology and Experimental Pathology, University of California, Berkeley, California.*]

I wish at this time to correct a mis-statement regarding the fermentative power of *B. histolyticus* that appeared in my 1922 paper,[3] in which I recorded acid and gas production in glucose. and uncritically accepted the records of Henry[4] and the British Medical Research Committee[5] of fermentation of glucose, levulose and maltose, which were based, like my own, on the study of a single strain. My own result may have been due to an undetected contamination. At any rate, Weinberg and Seguin[6],

1 Hall and Peterson, *Jour. of Bacteriology* (in press).

2 Hall, *Jour. Am. Med. Assn.*, 1919, lxxii, 274.

3 Hall, *Jour. Inf. Dis.*, 1922, xxx, p. 445.

4 Henry, *Jour. Path. and Bact.*, 1917, xxi, 344.

5 British Medical Research Committee, Report No. 39, 1919.

6 Weinberg et Seguin, *La Gangrene Gazeuse, Masson et Cie*, Paris, 1917.

Kendall, Day and Walker[7] and Kahn[8] were inclined to deny fermentation of sugars, and a more critical study of our five strains shows clearly that they ferment neither glucose, levulose, maltose, lactose, saccharose, salicin, glycerol nor inulin. That is, there is neither increase of hydrogen ion concentration nor considerable gas production.

B. histolyticus is thus the third sporulating anaerobe failing to derive its carbon from any of the commonly tested carbohydrates, glucosides or alcohols, the other two being *B. tetani* and *B. putrificus*.

The following table summarizes the outstanding differences between these three non-fermentative species of sporulating anaerobes:

	Morphology of spores	Tyrosin crystal	Culture filtrates
B. histolyticus	Subterminal, oval	Formed	Lytic
B. tetani	Terminal, round	Not formed	Non lytic but powerfully tetanospastic
B. putrificus	Terminal, round	Not formed	Harmless

249 (2209)

A note on the mechanism of the peculiar lesions produced by bacillus histolyticus.

By IVAN C. HALL and EMELIA PETERSON.

[*From the Department of Bacteriology and Experimental Pathology, University of California, Berkeley, California.*]

B. histolyticus was first described by Weinberg and Seguin[1] in 1915 as an obligate anaerobe from war wound infections in which is may display a remarkable and peculiar lytic activity. Pure virulent cultures injected intramuscularly into guinea pigs literally digest the flesh from the bones, hence the name—histolyticus.

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[7] Kendall, Day and Walker, *Jour. Inf. Dis.*, 1922, xxx, 141.

[8] Kahn, *Jour. Med, Res.*, 1922, xliii, 155.

*Weinberg et Senguin, *Comptes rend. Acad. des Sc.*, 1916, clxiii, 449.

Notwithstanding its marked ability to dissolve living muscular tissues its proteolytic action in brain, meat, milk, serum, and egg mediums is slow though fairly complete over long periods of time. Weinberg and Seguin[1] found that sterile filtrates liquify coagulated egg white and gelatin and Blancet Pozerski[2] and Dernbÿ and Blanc,[3] concluded that a tryptic enzyme is secreted by this species.

Weinberg and Sequin[1] also noted that sterile filtrates injected intramuscularly in fairly large doses produce large hemorrhages, apparently by destruction of the vascular walls and we have confirmed this phenomenon by injecting guinea pigs with 2 c.c. of the Berkefeld filtrate from 24 hour glucose broth cultures of several of our strains including the California strain. Sterile hematomas the size of a pigeon's egg generally appear within several days following such injections. The blood contained therein does not become hemolysed and recovery follows their drainage. We have never observed the muscular tissues to disintegrate or the skin to break, however, from the injection of filtrates as after the injection of living cultures. It seems apparent that the hemorrhage induced by the soluble secretions of this species plays an important rôle in the peculiar phenomena accompanying at least experimental infection in affording a suitable focus for the growth of this germ which appears rarely if ever to invade the blood stream itself.

1 Weinberg et Senguin, *La Gangrane Gazeuse, Masson et Cie*, Paris, 1917.
2 Blanc et Pozerski, *Comptes rend. Soc. Biol.*, 1920, lxxxiii, 1343.
3 Dernby and Blanc, *Jour. of Bact.*, 1921, vi, 419.

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250 (2210)

Growth and reproduction of rats on whole milk as the sole diet.

By LEROY S. PALMER and CORNELIA KENNEDY.

[*From the Section of Animal Nutrition, Division of Agricultural Biochemistry, University of Minnesota, St. Paul, Minn.*]

Several investigators have observed the effects of rearing rats on milk diets. Matill[1] and co-workers especially have studied this problem, using dried milk or concentrated solutions of milk powder for the most part in their experiments.

We have made a limited study of this problem with the following results. Using fresh, raw liquid milk exclusively[2] we have successfully raised 3 male albino rats to full size at maturity, beginning at weaning. This trial was begun August 10, 1922. Each rat was kept in a separate cage with one-quarter inch mesh wire bottom without bedding. The consumption of milk solids by these rats has varied between 35 and 100 grams per week per rat. These rats, however, on repeated trials have failed to exhibit any mating instinct when placed with females from our breeding colony, which were known to be in heat.

In a second experiment begun October 24, 1922, two colonies of rats, each containing 5 rats, with both sexes represented, on wire bottom cages, have not attained the expected size at maturity on the fresh, raw, whole milk diet. Growth was normal for the first 50 to 70 days only. The calculated average consumption of milk solids for each rat has not been as high as in the case of the rats in Experiment 1, kept in separate cages. The females have so far been entirely barren, confirming Mattill's results.

In a third experiment 3 female rats reared to partial maturity on mixed diet, each having reared one litter, were placed in

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[1] *J. Biol. Chem.*, 1920, xliv, 137-157; 1923, lv, 443-455. The literature is thoroughly reviewed in these papers.
[2] Our rats, however, had access to distilled water containing iodin.

the above colonies on the whole milk diet. To date, one of these rats has littered twice and the other two once, since being placed on the milk diet; for two of these litters the sire must have been one of the male rats raised on the milk diet, but the other two litters may have been sired by a male placed on the milk diet from a mixed ration.

The litters born in this experiment died within a day or two except in two cases. In one case the mother successfully nursed 4 rats on being given a yeast pellet[1] daily in addition to the milk. These 4 rats are now growing rapidly on the milk diet without yeast, but for a period of time were almost hairless. In the other case the mother rat is at present apparently successfully rearing a litter of 6 on addition of 0.2 gram daily of alcohol-extracted[2] yeast, indicating that the deficiency in the milk is not that of vitamin B.

One inference which it has seemed permissible to draw from Mattill's experiments is that the failure to secure normal growth on milk only is due in part to an improper balance between the food constituents. This inference does not seem to be substantiated, however, by a fourth experiment carried out by us in which a colony of 7 rats (3 males and 4 females) have grown normally[3] on an "artificial" dry milk composed of

Casein	18.7
Lactalbumin	3.1
Alcohol-soluble protein	0.5
Butter fat	28.7
Ether extract of alcohol-washed casein	0.5
Protein-free milk	48.5

This mixture contains the essential ingredients of cow's milk in the approximate proportions as they are secured from whole milk. To our surprise three of the females in this colony have littered once and two have littered twice.[4] The first litter of

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1 The yeast was a dried whole yeast for which we are indebted to the Northwestern Yeast Company, of Chicago. The amount given daily varied between 0.2 gram and 1 gram and probably averaged 0.5 for the nursing period.

2 The dry whole yeast was extracted for 24 hours with hot 80 per cent. alcohol in a Soxhlet type of extractor.

3 A cage with wire bottom of one-quarter inch mesh was used.

4 The second litters were sired by males on normal diet, it being necessary to reduce the colony because of shortage of some of the ingredients of the ration.

each rat thrived normally until about the 15th to 21st day when the young began to exhibit weakness, with spasms, and were destroyed by their mothers.   In the case of the second litter this result was prevented by giving one mother 0.2 gram of dry whole yeast daily and the other mother 0.2 gram of dry alfalfa meal in the form of a pellet.   Five of these second generation rats, representing both litters, are at present doing well on the "artificial" milk," without the additions mentioned.

It is not possible, at present, to reconcile the results of this experiment with those of Mattill or with our own on whole milk.   There seems to be evidence that milk only as the sole diet lacks something for the attainment of normal sexual maturity in the female rat.   Our results might also be interpreted to indicate a lack of something required for normal lactation, were it not for extensive unpublished data which we have showing that the addition of 10 c.c. of whole milk (1.25 grams of milk solids) to the diet of rats which fail to grow normally, as well as reproduce, has resulted in the securing of normal rats in experiments which have, to date, reached the third generation.

251 (2211)

The effect of local anaesthetics upon the conjunctivitis caused by mustard oil.

By ARTHUR D. HIRSCHFELDER.

[*From the Department of Pharmacology, University of Minnesota, Minneapolis, Minnesota.**]

Spiess[1] and Ninian Bruce[2] have shown that the oedema of the conjunctiva which can be produced by instilling mustard oil into the conjunctival sac of a rabbit, can be prevented by the instillation of 10 per cent. cocaine.   Bruce interprets this action as being due to the inhibition of vasdilator axon reflexes to the capillaries

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1 Spiess, G., *Muenchen Med. Woch.*, 1906, 345.
2 Bruce, A. Ninian, *Arch. f. Exper. Path. u. Pharmakol.*, 1910, lxiii, 424.

and smaller arterioles, and he found that if the ophthalmic nerve was cut before applying mustard oil oedema could then be produced only during the period in which the vasodilator fibers were still undegenerated, and a sufficient time were allowed for the vasodilator nerves to degenerate completely before the mustard oil was applied, oedema did not result after the instillation of mustard oil. Bruce's results have been confirmed by Bardy.[1]

In 1917 I[2] was able to demonstrate the following facts: (1) That the application of epinephrin, producing a local vaso constriction of the conjunctival vessels, inhibited the development of the oedema, for about an hour, but that after the epinephrin effect had worn off oedema developed subsequently. (2) That any continued lowering of the general blood pressure to less than 50 mm. Hg. prevented or greatly retarded the development of the oedema, even if the conjunctival vessels were dilated by the local administration of 1 per cent. sodium nitrite or if lymph secretion were increased by the intravenous injection of "Witte's peptone." (3) That intravenous lowering of the general blood pressure by the continuous intravenous injection of dilute hydrochloric acid, in spite of the acidosis produced, diminished or entirely prevented the development of the oedema from mustard oil. (4) That ligation of the carotid artery greatly reduced the development of oedema in the eye upon the side corresponding to the ligated artery; and that in the peripheral end of the ligated artery the blood pressure was less than 40 mm. Hg.

These experiments would lead to the conclusion that the oedema develops only when there is a sufficiently high pressure in the arterioles and capillaries to bring about a sufficient filtration after the walls of the blood vessels have been injured; and that if an adequate pressure is not present, the oedema does not develop whether the smaller vessels are dilated or not.

Since Krogh[3,4] in his studies upon the capillary circulation lays a great deal of emphasis on the functional importance of axon reflexes and upon Ninian Bruce's studies, it seemed of interest to determine the effect that local anæsthetics other than

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1 Bardy, H., *Skand. Arch. f. Physiol.*, 1914-15, xxxii, 198.

2 Hirschfelder, A. D., *Jour. A. M. A.*, 1916, lxvii, 1891; and *Trans. Sec. Pharmacol. and Therap. A. M. A.*, 1917, 182.

3 Krogh, A., *Jour. Physiol.*, 1919-1920, liii, 398; 1921, lv, 412.

4 Krogh, A., Harrop, G. A., and Rehberg, P. B., *Ibid*, 1922, lvi, 179.

Rabbit	Anæsthetic		Treated Eye	Reaction	Control Eye
1	Cocaine		No œdema		Oedema
2	Cocaine	10 min. before mustard oil.	Oedema		Oedema
3	Cocaine		Oedema		Oedema
4	Cocaine		Oedema		Oedema
5	Procaine		Slight œdema		Marked œdema
6	Procaine	10 min. before mustard oil.	Marked œdema		Less œdema
7	Procaine			Same in both eyes	
8	Procaine			Same in both eyes	
9	Saligenin		Marked œdema		Oedema
10	Saligenin	10 min. before mustard oil.	Marked œdema		Oedema
11	Saligenin		Less œdema	Same in both eyes	Marked œdema
12	Saligenin				
13	Butyn			Marked œdema in both eyes	
14	Butyn	10 min. before mustard oil.		Marked œdema in both eyes	
15	Butyn			Marked œdema in both eyes	
16	Butyn			Marked œdema in both eyes	

cocaine might produce upon the development of oedema from mustard oil. We tested the effects of instilling the following solutions upon the development of mustard oil conjunctival oedema: (1) 4 per cent. cocaine solution in 0.9 per cent. NaCl; (2) 4 per cent. procaine (novocaine) in 0.9 per cent. NaCl; (3) 4 per cent. saligenin in 0.9 per cent. NaCl; and (4) 2 per cent. butyn

$$\overset{\diagup\diagdown NH_2}{\underset{\diagdown\diagup COOCH_2CH_2CH_2\, N(C_4H_9)_2,}{|}} \quad \text{sulphate}$$
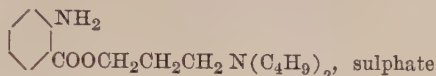
dissolved in distilled water.

These substances produced complete sensory anæesthesia of the conjunctiva so that the corneal conjunctiva would be scratched with a thin copper wire without causing any winking reflex or any other movements of the rabbit.

Procaine and butyn seem to have no marked effect on blood vessels; saligenin definitely dilates them.

It will be seen that the oedema developed in the same manner and degree and with the same rapidity in the anaesthetized as in the unanaesthetized eye, in all the experiments with the exception of one in which the oil was instilled almost immediately after the 4 per cent. cocaine. This exception may be due to some vaso constrictor action of the cocaine.

These experiments seem to indicate that mere sensory anaesthesia does not prevent or inhibit the development of oedema from mustard oil, and that the maintenance of a high filtration pressure in the vessels of the eyelid is the most important factor in the development of oedema in an area, the walls of whose blood vessels have been injured by this agent.

It is possible that in Bruce's employment of 10 per cent. alypine and 10 per cent. cocaine to inhibit the oedema, the hypertonic solution of the drug acted to constrict the vessels; and that, in his experiments on cutting the ophthalmic nerves, the still intact vasoconstrictor fibers may have brought about a predominant constrictor response after the vasodilator nerves had degenerated.

252 (2212)

Studies on quantitative determination of fat in micro-organisms.

By A. G. BENTON.

[*From the Department of Bacteriology, University of Minnesota,
Minneapolis, Minn.*]

Great difficulty is experienced in extracting fat from wet or
dried micro-organisms. It has been suggested that a large por-
tion of the fat they contain may be held in physical or chemical
combination by some ingredient of the protoplasm and various
preliminary treatments have been developed to free it from such
combination. Three of the simplest are that of Larson and
Larson for bacteria[1] which depends on simultaneous drying and
extraction by acetone, followed by ether extraction of the solid
residue and the acetone extract; I. S. MacLean's method used on
yeast[2] which consists in boiling with normal HCl, washing and
then extracting with ether in a Soxhlet; and the method devel-
oped by C. R. Smith for work on edible pastes, in which he boils
the sample with alcoholic ammonia, and then extracts with ether.

The work here reported was done on Oidium Lactis. In the
first experiment a two weeks' growth was drained by suction
and divided into three portions, one of which was treated by
each of the above methods without previous drying. The resi-
dues as well as the extracts were dried to constant weight and
the total dry weight of the samples obtained by addition. The
MacLean method is impractical on moist samples, as an unman-
ageable mucilaginous brown material results from the acid treat-
ment. The acetone method gave 1.21 per cent. ether extract;
the alcoholic ammonia method gave 6.13 per cent. No further
studies were made on the lipoids thus extracted, as drying to
constant weight, either in an oven at 100° or in a vacuum dessi-
cator over P_2O_5 at room temperature, results in a hard, semi-
transparent brown material, insoluble in petroleum ether, only a
portion of which is soluble in ethyl ether.

In another experiment, a three weeks old growth was spread

1 *Jour. Inf. Dis.*, 1922, xxxi, 407.
2 *Biochem. Jour.*, 1922, xvi, 370.

on fine linen, dried at 37° for two days, ground in a mortar until it would pass a 60 mesh sieve, and divided into four portions. The first was dried at 100° to constant weight which was 45.7 per cent. of its original weight. The second, without preliminary treatment was extracted in a soxhlet with ether then with alcohol, and again with ether. The ether extract of the alcohol extract was added to the other ether extracts and dried. The result was 7.48 per cent. of the original dry weight of the powdered mycellium. The third portion treated by the alcoholic ammonia method, gave 8.45 per cent.; the fourth portion treated by the HCl method gave 10.13 per cent. An aliquot part of this last sample, which was not dried, but was saponified with alcoholic potash yielded 38.7 per cent unsaponifiable matter.

253 (2213)

Some observations on pellicle formation.

By A. G. BENTON.

[*From the Department of Bacteriology, University of Minnesota, Minneapolis, Minn.*]

Bacillus subtilis characteristically produces a diffuse turbidity on broth, which usually clears toward the end of the first twelve hours, the organisms floating on the surface in small islands. These later grow together producing a heavy wrinkled pellicle. It seemed therefore that a study of possible factors influencing this spontaneous migration to the surface might illuminate the subject of pellicle-formation and surface growth in general.

Equal amounts of a young diffuse culture of B. subtilis were introduced into tubes containing each 10 c.c. of ordinary broth. These were incubated and at hourly intervals for 36 hours observations were made of morphology, progress of growth, spore formation, buoyancy of the pellicle and surface tension of the medium. The surface patches appeared at 10 hours, and the pellicles were well formed at 15. The first positive heat test for spores was obtained at 20 hours, but the cultures were not con-

sistently positive until after the 24th observation. At the 25th hour the first spores were observed in stained preparations. At all times the majority of the growth could be centrifuged to the bottom of the tube, showing that the organisms are heavier than the medium. Occasionally small rumpled fragments of the pellicle remained at the top of the centrifuge tube, but these may well have imprisoned air bubbles.

Larson[1] has shown that this organism does not form pellicles on media whose surface tension has been sufficiently reduced by soap. The question naturally arises whether the diffusely growing organisms exhaust some surface tension depressant in the media prior to their upward migration. This, however, is not the case, since the surface tension remained approximately 59 dynes throughout the period of hourly observation (36 hours) and was still the same 13 days after inoculation, at which time the pellicles sank spontaneously.

The avidity which this organism is supposed to have for oxygen, is apparently not a factor, since cultures in large flasks with oxygen bubbled up continuously from the bottom still exhibit surface growth.

A significant observation is that the upper surface of young pellicles is not wetted by water, although ethyl ether, petroleum ether, and chloroform spread upon it readily. It is impossible to invert a floating pellicle. If one lifts it and attempts to replace it upside down on the water it at once rights itself and continues to do so, until repeated manipulation has injured and wetted the surface originally uppermost. In such a case the pellicle usually slowly sinks. Experiments are now under way to determine whether this behavior is due to an accumulation of old cells storing lipoids in the upper layers of the pellicle.

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[1] *Jour. Inf. Dis.*, 1919, xxv, 1.

254 (2214)

Studies on the physiology of the liver. VII. The effect of insulin on the blood sugar following total and partial removal of the liver.

By FRANK C. MANN and THOMAS B. MAGATH.

[*From the Division of Experimental Surgery and Pathology, The Mayo Foundation, and Section on Clinical Laboratories, Mayo Clinic, Rochester, Minnesota.*]

Previously we have shown that the total removal of the liver is followed by (1) a rapid decrease in blood-sugar, (2) always accompanied by a characteristic group of symptoms, and (3) the administration of glucose which abolishes the symptoms, temporarily restoring the animal to the normal state. All of this is comparable to what happens when a large dose of insulin is administered to a normal animal.

The problem investigated is the rôle the liver plays in the effect of insulin. Therefore, insulin was administered before and after the total removal of the liver in dogs. Removal of the liver did not affect the sharp precipitate drop in the blood-sugar after administration of insulin. Whereas injecting glucose in an animal with its liver intact during the condition of insulin hypoglycemia restores the sugar level permanently, after the liver is removed no permanent level can be maintained despite frequent large doses of glucose.

The same experiments were carried out on dogs before and after partial removal of the liver. While the same sharp drop in blood-sugar occurs after giving very small doses of insulin, there is a slow restoration of the curve, even though only 20 per cent. by weight of the liver remains.

From these experiments we conclude that the symptoms associated with hypoglycemia following the administration of insulin do not differ essentially from those we noted in association with the total removal of the liver, and the action of glucose seems to be identical in the two conditions. The effect of large doses of insulin in producing hypoglycemia is not changed by the total removal of the liver, nor is the hypoglycemic action of small doses of insulin modified by partial (60 to 80 per cent.) removal of this organ. However, the liver is necessary for the permanent restoration of the sugar level.

ABSTRACTS OF COMMUNICATIONS.

Seventh meeting.

Western New York Branch.

Ithaca, New York, May 12, 1923.

255 (2215)

The effect of thyroid gland from young calf upon the blood sugar in depancreatized dogs.

By G. A. FRIEDMAN.

[*From the Department of Clinical Pathology, College of Physicians and Surgeons, Columbia University.*]

Every clinician must have observed from time to time the appearance of sugar in the urine after prolonged use of thyroid extract in patients.

Magnus-Levy[1] writes that glycosuria may be noted from large dosages of thyroid in man as well as in dogs. Since glycosuria may occur as a result of thyroid feeding, it was thought that the high percentage of iodine in the gland of the ox might be the determining factor in the appearance of sugar in the urine after prolonged use of the drug.

According to Hammarsten[2] the thyroid of man contains 0.34 per cent. iodine; the thyroid of ox contains 0.86 per cent. It was thought that thyroid which is iodine free must have the reverse effect on the blood-sugar contents. It is a well established fact that the thyroid of newborn animals does not contain iodine. Thyroids from such calves were procured from the slaughter house immediately after killing and extracts from the weighed glands prepared. The alcoholic extracts in Ringers solution were injected intravenously or subcutaneously or administered by the stomach tube to dogs. The extracts were prepared in the same manner as the pancreatic extracts by Banting and Best.

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1 Magnus-Levy, Kraus und Brugsch, *Specielle Pathol. u. Therapie*, 1919, i, 8.

2 Hammarsten, ''Physiological Chemistry,'' 1909, Fifth Edition, John Wiley and Sons, New York. English translation by John A. Mandel.

After weighing the thyroids they were cut in very small pieces and placed in 0.2 per cent. HCl in 95 per cent. alcohol and allowed to stand for two days. They were then macerated with quartz and filtered, or when using large amounts first macerated and then put in a press and filtered. The clear filtrate was evaporated to dryness in a warm air current. On the next day or on the third day the dry residue was emulsified with 25 c.c. Ringers solution. 25 c.c. of this solution contained the thyroid residue from 10, 20 or 50 grams gland.

The blood-sugar was determined before injection or oral administration and two or three hours later. The dogs were starved at least 24 hours before the experiments. For one experiment a normal dog was injected first with calf's pancreas prepared by the same method.

	Dog 168. Male. Weight, 8 kilos.
January 3, 1923	Bloodsugar 89 mg. in 100 c.c.
January 8, 1923	Bloodsugar 89 mg. 12 M.
January 8, 1923	Bloodsugar 45 mg. 2 P. M. after injection of 5 c.c. residue from whole pancreas.
January 26, 1923	Bloodsugar 100 mg. 12 M.
January 26, 1923	Bloodsugar 80 mg. 2 P. M. after intravenous injection of 10 c.c. Ringers solution which contained a residue from 8 grams thyroid gland.
Control—	
January 28, 1923	Bloodsugar 98 mg. 1:30 P. M.
	Bloodsugar 100 mg. 3:30 P. M. after intravenous injection of 10 c.c. Ringers solution *per se.*
	Dog 169. Male. Weight, 12.2 kilos.
January 10, 1923	Partial pancreatectomy. Glycosuria absent.
January 12, 1923	Bloodsugar 133 mg: 2 P. M.
	Bloodsugar 123 mg. 4 P. M. after intravenous injection of 10 c.c. Ringers solution which contained residue from 4 grams thyroid.
January 13, 1923	Bloodsugar 128 mg. 2:15 P. M.
	Bloodsugar 117 mg. 4:15 P. M. after subcutaneous injection of 10 c.c. Ringers solution which contained residue from 4 grams thyroid.
	Dog 175. Male. Weight, 11.6 kilos.
March 10, 1923	Bloodsugar 80 mg. 12:30 P. M.
	Bloodsugar 71 mg. 3:30 P. M. after administration through stomach tube of 10 c.c. Ringers solution which contained residue from 4 grams thyroid gland.
	Dog 171. Male. Weight, 19.8 kilos.
January 31, 1923	Total pancreatectomy.
February 15, 1923	Incomplete thyroidectomy. Persistent intense glycosuria until March 7, when disposed of.
February 7, 1923	Bloodsugar 333 mg. 2:25 P. M.
	Bloodsugar 312 mg. 4:25 P. M. after oral administration of 10 c.c. Ringers solution which contained residue from 8 grams thyroid.

February 12, 1923   Bloodsugar 312 2:15 P. M.
                       Bloodsugar 277 4:15 P. M. after oral administration
                           of 25 c.c. Ringers solution which contained residue
                           from 50 grams thyroid.

Control—   Bloodsugar 250 mg. 3:15 P. M.
February 23, 1923   Bloodsugar 250 mg. 5:15 P. M. after oral administra-
                           tion of 25 c.c. Ringers solution *per se*.

Dog 176.   Male.   Weight, 9 kilos.

March 22, 1923   Almost complete pancreatectomy.   Persistent intense
                           glycosuria.   Dog was found dead April 3, 1923.
April 2, 1923   Bloodsugar 164 mg. 2 P. M.
                       Bloodsugar 127 mg. 4 P. M. after oral administra-
                           tion of 25 c.c. Ringers solution containing residue
                           from 50 grams.

Dog 167.   Female.   Weight, 7.4 kilos.

December 21, 1922   Pancreatic ducts were ligated and cut.
January 25, 1923   Bloodsugar 93 mg.
                       Bloodsugar 89 mg. 1:30 P. M.
                       Bloodsugar 71 mg. 3:30 P. M. after oral administra-
January 29, 1923   tion of 10 c.c. Ringers solution containing residue
                           from 8 grams thyroid.

These experiments tend to show that there is in the normal as well as in the depancreatized dog a reduction in blood-sugar after intravenous, subcutaneous or oral administration of thyroid gland of young calf. It is evident from these experiments that extracts prepared from larger amounts of thyroid were more effective in reducing the blood-sugar contents than when prepared from smaller amounts. It must be admitted that the experiments are too few to draw any definite conclusions. The fact that the thyroid gland from newborn calves has a marked influence in lowering the blood-sugar contents in depancreatized dogs when given by mouth, is important since the oral administration of insulin has no effect on the blood-sugar contents.

Further experiments along these lines are in progress.

256 (2216)

A rapid method of preparing the anti-diabetic substance of pancreas.

By R. S. ALLEN, H. A. PIPER, C. P. KIMBALL and JOHN R. MURLIN.

[*From the Physiological Laboratory of the University of Rochester, Rochester, New York.*]

As reported[1] at the last meeting of this branch, heating to 75° and 80° C. for one-half hour does not destroy the anti-diabetic substance extracted in acid aqueous media. The original observation of Murlin and Kramer that a potent extract could be prepared by boiling dog's pancreas in 0.2N HCl was confirmed[2] some time ago. Recently we have compared the yield of rabbit units obtained on the one hand by grinding macerated pancreas in 0.2N HCl in a bacteria grinder for 15 hours and, on the other hand, by heating to 75° C. for one hour or bringing just to boiling temperature.

Kimball, Piper and Allen[3] showed also at the last meeting that the active substance is precipitated by complete saturation with sodium chloride, and with either of the alcohols, methyl, propyl, butyl or amyl when added to a solution in 80 per cent. ethyl alcohol. For example, preparation No. 140, heated in 0.2N HCl to 75° C, cooled, strained through cheese cloth, neutralized to P_H of 4.1, and filtered, gave in two tests representing 10 gms. pancreas each, a fall of blood-sugar in normal rabbits of 44 and 62 milligrams. The second day the filtrate was precipitated by saturation with sodium chloride, the precipitate taken up in 70 per cent. alcohol, centrifuged to remove insoluble protein and reprecipitated with 5 vols. amyl alcohol. The excess of amyl alcohol was evaporated off before a fan and the residue taken up in sterile water. Equivalent doses representing 10 grams pancreas now gave drops in blood-sugar in rabbits of the same size of 77 and 74 milligrams showing that all the potency had been re-

1 Piper, H. A., Mattill, H. A., and Murlin, J. R., PROC. SOC. EXP. BIOL. MED., 1923, xx, 413.

2 Murlin, J. R., Clough, H. D., Gibbs, C. B. F., and Stokes, A. M., *Jour. Biol. Chem.*, 1923, lvi, 253.

3 Kimball, C. P., Piper, H. A., and Allen, R. S., PROC. SOC. EXP. BIOL. MED., 1923, xx, 414.

moved. The filtrate from the NaCl precipitate contains no active substance. The rapid method which, even with filtration over night, can be carried to completion in 18 hours is as follows:

1. Beef pancreas trimmed free of extraneous tissue at the slaughter house is placed at once in 0.2 N HCl, chilled to 0° C., and is transported to the laboratory in this condition.

2. Upon arrival at the laboratory the acid is discarded, the pancreas hashed in a meat grinder and a known weight mixed immediately with 4 vols. fresh 0.2N HCl.

3. The mixture is brought to 75° C. where it can be held for one hour. Or it may be brought rapidly over a free flame just to the boiling point.

4. It is then chilled under the tap to 20° C. or lower to congeal the melted fat which is skimmed off.

5. The material is strained through cheese cloth and neutralized with N/1 NaOH to a P_H of 4.9 or titration to phenolphthalein to .01 N. It is then filtered through coarse filter paper over night.

6. To each 1,000 c.c. of filtrate 250 grams NaCl are added and stirred to complete solution. Precipitation is rapid and complete. The precipitate contains all the active substance together with some extraneous proteins.

7. After standing for at least two hours the suspended precipitate is decanted and either filtered or centrifuged off.

8. In either case the precipitate is treated with alcohol of not over 70 per cent. by volume and the insoluble proteins discarded.

9. Three to five volumes of amyl alcohol are added and the mixture thoroughly shaken and centrifuged. The precipitate is found between the aqueous and alcoholic layers.

10. The precipitate is treated with 80 per cent. alcohol, filtered, and the filtrate evaporated to dryness by air currents. Further purification is accomplished by re-solution in 80 per cent. alcohol and evaporation *in vacuo*.

11. The dry material is taken up in sterile water and filtered aseptically, adjusting the reaction of P_H of 4.0 or lower.

12. This final product usually gives a very faint biuret reaction.

The final product is water clear and, because the final precipitate is very soluble in water, can be made as concentrated as desired for administration.

For treatment of perfustates[1] made with 0.2 per cent. HCl and improved extracts recently prepared by percolation, neither of which contains much protein, the method of refinement yielding best results is as follows:

1. Excess of acid is neutralized to P_H of 5.85. Acid meta-proteins are thrown down. Fluid is filtered and filtrate immediately readjusted to P_H of 4.1.

2. Sodium chloride in the proportion of 1 gram salt to 3.5 grams pancreas used is added to the first filtrate and dissolved. The fluid is then evaporated to dryness.

3. Excess salt and proteins are left behind by successive fractional extractions with 80 per cent. alcohol and evaporation to dryness.

4. The final residue is treated with a small volume of sterile, distilled water and the reaction which is now about N/5 HCl, readjusted to P_H 4.1 (15 c.c. fluid = 2.1 c.c. N/10 NaOH to phenolphthalein). *The anti-diabetic substance is precipitated in a form which is insoluble in sterile distilled water but is readily soluble in weak acid or weak alkali.*

5. It is free of chlorides and gives none of the following reactions for proteins: Biuret, Millon's, Xantho-proteic and Hopkins-Cole.

257 (2217)

The fate of iletin in the animal body.

By G. W. PUCHER, K. F. CORI, and B. D. BOWEN.

[*From the Buffalo General Hospital, Buffalo, N. Y.*]

Fasting animals show a different counter regulation for iletin than animals which received food. Rabbits with food plus iletin may show lowering of the blood-sugar for periods of eight hours. Convulsions have been observed as long as 14 hours after the iletin injection. This indicates that iletin is not rapidly destroyed or eliminated by the animal body. No change in the tolerance for iletin was observed after a daily administration of the extract for 83 days.

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[1] Murlin, J. R., Clough, H. D., Gibbs, C. B. F., and Stone, Neil C., *Amer. Jour. of Physiol.*, 1923, lxiv, 348.

258 (2218)

The free sugar in the liver and its significance for carbohydrate metabolism.

By K. F. CORI, G. W. PUCHER, G. T. CORI.

[*From the State Institute for the Study of Malignant Disease, Buffalo, N. Y.*]

Curves were presented showing the influence of adrenaline and iletin on the free sugar content of the liver and the blood-sugar. Further curves showed the influence of glucose ingestion on the free sugar of the liver and the relation of free liver sugar to glycogen synthesis with and without iletin. These curves show that the prolonged hyperglycemia after adrenaline injection is due to the fact that the sugar set free in the liver during the first hour diffuses into the blood stream very slowly. It was shown that iletin lowered the free sugar of the liver even during glucose absorption. Ingestion of glucose without iletin increases the free liver sugar. Glycogen synthesis without the administration of iletin has only been observed when the free liver sugar was above its normal value. (Normal value for animals starved for 24 hours is 0.3-0.35 per cent.) Glycogen synthesis under the action of iletin occurs from normal or even lower than normal free sugar levels. This data indicates a disturbance of the ferment equilibrium normally established in the liver and is one of the factors involved in the lowering of the blood-sugar observed after the injection of iletin.

259 (2219)

The determination of galactose in the presence of glucose.

By K. F. CORI, G. W. PUCHER, G. T. CORI.

[From the State Institute for the Study of Malignant Disease, Buffalo, N. Y.]

Folin and Wu's method for blood-sugar has been adapted to the determination of galactose glucose mixtures. The method is based on the fact that copper solutions of different alkalinities give different reduction values for galactose, but the same for glucose. It was also found that Bertrand's as well as the Myer-Benedict method give the same reduction values for glucose and galactose. The use of the latter methods in combination with that of Folin and Wu is unsatisfactory due to errors that may be introduced by the discrepancies for glucose values obtained on the same blood.

260 (2220)

Energy metabolism of premature infants.

By M. ELIZABETH MARSH (by invitation).

[From the Physiological Laboratory of the University of Rochester, and the Obstetrical Division of the Highland Hospital, Rochester, N. Y.]

Energy metabolism studies have been made in 82 periods of observation upon 21 prematurely born infants including those previously reported.[1] Both the oxygen and carbon dioxide, and thus the R. Q., were detemined for each separate period and heat calculated from the oxygen consumption. Temperature within chamber was usually kept between 24° C. and 28° C. Infant was fed (amount of feeding determined by weighing before and after), placed in the chamber and a preliminary period run to allow a lapse of 30 minutes between time of feeding and the beginning of the first period. An average minimal heat production per sq. m. per hour (Lissauer formula) of 25.0

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1 Murlin and Marsh, PROC. SOC. EXP. BIOL. AND MED., 1922, xix, 431.

calories on infants over 24 hours of age was obtained as compared with 26.75 calories obtained by Benedict and Talbot[1] for normals from 2 to 8 days of age. These observers found a rather constant heat production from second to seventh day, the average of all basal periods being 27.87 cal. per sq. m. per hr. as compared with 25.72 calories for our prematures.

In comparing maximum with minimum heat values for each infant an average of 44.5 per cent. increase was obtained. Percentages varied from 5.7 with slight restlessness to 88.77 with hard crying 36 minutes (¾ of the period). Comparing the increases obtained in succeeding periods of the same observation in which all the factors, except the activity, were the same, an average of 16.5 per cent. was obtained. Increases varied from 2.5 per cent. with very slight restlessness to 40.5 per cent. with crying 16 minutes of a 33-minute period. Minimal metabolism in children of comparable ages averages 31 cal. per sq. m. per hr. after feedings of from 60 to 84 grams breast milk and 24 cal. after feedings of from 25 to 35 grams.

261 (2221)

A test for peristaltic activity.

By PIERRE A. FISH.

[From the Cornell University, Ithaca, New York.]

The agent used is the red dye known as Sudan III. Its advantages are that it may be used in very small amount thus interfering very little, or not at all, with the normal processes in the alimentary tract. The amount used for man is from 50 to 70 milligrams (¾ to 1 grain). It is soluble only in a fatty medium or in fat solvents. Taken with, or just after a meal, it is not impossible that a slight amount may be combined with the fat of the meal and absorbed to a slight extent; otherwise it is not, apparently, susceptible to the alimentary secretions. The tests have not been interfered with by any absorption that may have occurred. The red color of the dye is essential for the test.

The rapidity with which the dye, mixed with the ingesta, will

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[1] Benedict and Talbot, Carnegie Institute Pubs., No. 233.

pass through the intestinal tract will depend upon peristaltic activity, the secretions, the frequency of evacuations and the amount of material ahead of it. In constipation the time will be lengthened and in diarrhea it will be shortened.

The test consists in giving 50-70 miligrams (¾ to 1 grain) of Sudan III in a gelatin capsule, just before, or after, a meal. One or two grams of the feces from the subsequent evacuations are taken and dried. A record should be kept of the time of administration of the dye and of the evacuations which follow. After drying, the material is extracted with ether. The extract from the first feces passed (before the dye appears) is used as a control. Its color is usually greenish yellow or light brown. Later evacuations, depending on conditions, begin to show a red color. If the tests be continued long enough, the maximum red color will be obtained which will decrease, in later evacuations, until the normal color is again reached.

Three experiments upon the same individual gave the first appearance of the dye in 17, 15 and 25½ hours respectively. The maximum color was obtained in 17, 38½ and 51 hours. The return to a normal color occurred after 52½ hours (4 evacuations), 65¾ hours (6 evacuations), and 73 hours (5 evacuations).

In another experiment, in which the subject had diarrhea, the dye appeared in the excreta 4½ hours after it was administered. The first evacuation occurred 15 minutes after the dye had been taken and was used as the control. The maximum color was reached in about 11½ hours and the normal color was reached in about 48½ hours (7 evacuations).

Seventy milligrams of the dye were given to a poodle dog just after his bowels had moved. There was another evacuation 3¼ hours later, just before the animal was killed. The postmortem showed that the small intestines were practically empty except at the ileum, near the cecum. The large intestine contained a fair amount of material. Samples of the contents were taken from the stomach and at 30 centimeter (1 foot) intervals along the intestinal tract. A satisfactory test was obtained from the gastric contents, showing that a fair proportion of the dye was still present. The samples from the small intestine showed only faint reactions because of the scanty amount of material present —that from the duodenum was practically colorless. The sample from the ileum gave a good reaction because of the increased

amount of material present. The samples from the rectum and colon gave the most marked reactions of any, thus showing the presence of a considerable proportion of the dye. The intestinal tract was measured and it was determined that the dye had passed through it at the rate of 1.2 centimeters per minute. The dog evidently had diarrhea and the passage of a considerable proportion of the dye through the length of the intestines in $3\frac{1}{4}$ hours is relatively rapid.

Earlier experiments upon the cow and goat, under normal conditions show that the minimum time for the passage of the dye is 16-17 hours for the former and 14-17 hours for the latter. The uniformity in the minimum time of passage in widely separated species of animals is remarkable, when the difference in habits, food eaten, and the length of the alimentary tract is considered.

The minimum time is tabulated as follows:

Man ....15 —25 hours
Cow ....16 —17 hours
Goat ....14 —17 hours
Horse ....$15\frac{1}{4}$—20 hours (Cuguini, powdered Brazil nut)

The ratio of the body length to the length of the intestinal tract is given as follows:

Man ....1 —10 (Legs not included in body length)
Horse ....1 —12 (Colin)
Cow ....1 —20 (Colin)
Goat ....1 —27 (Colin)

The average length of the intestinal tract of man is reckoned at 30 feet. From figures taken from Colin, who has computed the length of the intestinal tract in the domesticated animals, that of the horse is $3\frac{1}{4}$, the goat $3\frac{1}{2}$ and of the cow 6 times longer than that of man.

The fact that the herbivorous animals normally evacuate their bowels more frequently than man during the 24-hour period is a factor to be considered.

The test is simple, easy to carry out and the Sudan III has apparently no irritating or injurious effect upon the bowels. In the absence of an X-ray apparatus, much important information may be obtained. It would seem to be a useful method in arriving at a more positive diagnosis relative to certain intestinal disorders, such as torpidity, intussusception, strangulated hernia, impaction, or where any obstructive cause might be suspected.

262 (2222)

An investigation as to the etiology of azoturia.

By C. E. HAYDEN.

[From the New York State Veterinary College at Cornell University, Ithaca, New York.]

Azoturia is a disease affecting horses. There are various synonyms of which "Hemoglobinuria" and "Monday Morning Sickness" are common. It is most common in working horses. Animals that are worked hard during the week and allowed to stand in the stable over the week end without exercise and without any decrease in the ration given on working days are most often affected. When exercise begins they are apt to show stiffness and lameness in muscles of the hindquarters. Paralysis of quarters is common and animals get down. They often die as a result of the changes having taken place in the tissues and in spite of any treatment.

The disease has been attributed to several different causes such as myogenic autointoxication, the formation of an hemolytic ferment, a very profuse formation of urea and other extractives, an abnormal amount of dextrose producing a myositis and secondary nephritis. Uremic poisoning is also given as a cause. Much emphasis has been laid upon the abundance of extractives either as a result of tissue changes or as a product of digestion.

Average readings in mgms. per 100 c.c. of blood for normal horses and four cases of azoturia reported in 1921 as well as twelve cases of azoturia from which samples were obtained in 1922-1923 and not as yet reported are appended.

1921	Total non-protein nitrogen	Urea	Uric acid	Pre-formed Creatinine	Sugar
Normal	33.9	18.71	2.45	1.81	102.9
Blood at height of azoturia	30.	18.8	2.3	1.7	102.6
Blood at recovery	28.5	17.1	2.31	1.87	88.2
Ave. twelve cases of azoturia 1922-23	38.34	16.79	2.81	1.74	121.

In the data submitted blood-sugar shows the greatest increase. It is well to bear in mind that excitability is a marked symptom in many cases and such a state often indicates a hyperglycemia *per se.* The average increase in the extractives does not seem large enough to indicate that the increase is any more a cause of the disease than it might be a result of the tissue activity taking place.

Urine examinations do not indicate any more nephritis than might be expected to accompany such violent pathological changes in the tissues as take place in axoturia. Hemoglobinuria is so characteristic that the urine of typical cases is coffee colored rather than the color normal to the urine of the horse.

A German, Dr. Hertha, has recently advanced the theory, with what he considers abundant proof, that the condition is due to excessive lactic acid formation in the tissues. Dr. P. A. Fish, Dr. J. N. Frost and the author are now working on this theory and hope to report results in the near future.

263 (2223)

Further studies on "posterior paralysis" in swine.

By L. A. MAYNARD, S. A. GOLDBERG, K. V. WILLIAMS and O. B. CHRISTY.

[*From the Department of Animal Husbandry and the Department of Comparative Pathology, Cornell University, Ithaca, N. Y.*]

"Posterior paralysis" is one of the terms rather loosely used to designate a trouble in swine which is characterized by stiffness, particularly in the hind legs, inability to rise or stand, the occasional swelling of the joints and fractures of the leg bones. Some preliminary studies relative to this trouble were made the subject of a previous communication.[1]

In continuation of these studies, 24 pigs, around 30 pounds in weight, were placed on experiment in the summer of 1922. The characteristic symptoms were produced on a ration of yellow

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[1] Maynard, L. A., PROC. SOC. EXP. BIOL. MED., 1922, xix, 427.

hominy, middlings, and casein, made into a slop with an equal weight of skim milk. A check group receiving added minerals in the form of calcium carbonate and bone meal did not develop the trouble. The alleviation of stiffness and the resumption of growth was brought about in the group receiving no extra minerals either by the addition of the minerals in question, or, by a daily addendum of 10 c.c. of cod liver oil.

In the following winter eight out of thirteen pigs developed the symptoms on the same ration, containing, however, yellow corn meal in place of hominy,—even when calcium carbonate and bone meal were included. Orange juice was added to the ration of three of the affected pigs, which were obviously near death. There was a marked temporary improvement followed in two weeks by a decline and death. The addition of cod liver oil produced only a slight improvement over a three weeks' period. When the cod liver oil was replaced by 5 per cent. of chopped alfalfa hay in the case of two of these pigs, permanent improvement resulted.

According to the symptoms of apparent paralysis, it was thought that the lesions might be in the lumbar enlargement of the spinal cord. Examination of the cord by the Nissl's method, did not show any tissue changes. The principal and constant lesions in the pigs fed as described in the preceding paragraph were found in the bones and in the kidneys. Eight of the pigs examined showed acute pneumonia. The bronchi of all of these were infested with *strongylus paradoxus*. The bone lesions were most frequently seen in the ends of the femur. Here the epiphyseal cartilage was thicker than normal and irregular. The sub-epiphyseal bony trabeculæ were atrophied and gone in places, with the marrow spaces filled with fibroblasts, newly formed blood vessels, and hemorrhage, mostly under the epiphyseal cartilage. As a result of this, the cartilage was loosened from the diaphysis in some of the animals. The subchondral epiphyseal bone showed similar changes, consisting of granulation tissue and hemorrhage in place of normal bone. The zone of provisional calcification was irregular, the articular cartilage was degenerated in places and in other areas was invaded by blood vessels from the subchondral bone. The epiphysis was affected for two-thirds of its extent. These changes easily account for the spontaneous fractures encountered in two of the pigs. At the junction of the costal cartilage, there was a thickening in most of the cases ex-

amined.   There was irregularity in the calcification, with the
spaces filled with granulation tissue, hemorrhage, and separated
areas of degenerated cartilage cells.   There were what appeared
as subperiosteal hemorrhages on the pleural surface of the ribs
in one pig.   Histologically, however, there was in this area a
proliferation of tissue with apparent formation of osteoid tissue.

The kidneys showed hyperemia of the glomeruli, albumin casts
in the convoluted tubules with slight cloudy swellings, dilation
of the collecting tubules, with lymphocytes and fibroblasts be-
tween the tubules in the zona radiata of the cortex.   Routine
clinical examinations of the blood showed no leucocytosis and
no anemia.   The dilation of the collecting tubules in the kidneys
was most likely due to retention of urine for long periods, on
account of inability to rise.   The renal condition suggested a pos-
sibility that the animals were poorly housed.   The fact that the
condition was corrected by feeding alfalfa shows that the etiol-
ogy is to be looked for in the diet.

It is quite evident that lack of minerals was concerned with
the development of the trouble in the summer of 1922, and the
fact that cod liver oil as well as added minerals cured, suggests
rickets as the specific trouble.   On the other hand, the large num-
ber of cases of trouble produced in the winter of 1922-23, de-
spite added lime and phosphorus, indicates that a mere lack of
them is not the only factor involved.   The constancy of hemor-
rhage found on histological study suggests the possibility of
scurvy being concerned.   However, the bone lesions also indi-
cated a deficiency of calcium assimilation suggestive of rickets.
It seems possible that both of these specific diseases may be con-
cerned in the external symptoms noted.

264 (2224)

Observations on the epidemiology of pemphigus neonatorum. (Impetigo contagiosa bullosum?)

By RALPH R. MELLON, WILLARD S. HASTINGS and DOROTHY W. CALDWELL.

[*From the Highland Hospital, Rochester, New York.*]

On account of recrudescences of this condition in obstetric wards following long periods of quiescence the presumption obtains that the infection has never been completely eradicated from the institution. In order to verify this conjecture, attention has been concentrated on the sporadic case, especially that one occurring after months of freedom from the disease.

Careful study of such a case reveals the interesting fact that the origin of the disease in the infant was apparently by way of the mother's milk, which was heavily infected with pure hemolytic staphylococcus aureus and albus, the former predominating. The lesion on the infant's skin followed forty-eight hours after a rather generalized rash on the mother. It was of note that aside from this macular type of rash the mother was quite normal. The rash itself resembled that often caused by drugs. Cultures of the cervix blood and stool were negative for the infecting organisms, but the urine contained a few colonies. The first and last portions obtained by completely emptying the breasts with a sterile breast pump contained approximately equal numbers of the organisms, which were quite abundant, although no evidence of mastitis was present, either clinical or from examination of the milk.

Although ingesting large numbers of the organism in the milk, the infant's stools were negative for the specific germ, which ruled out the possibility of the initial thigh lesions being the result of direct contamination from the stool. Many lesions appeared on the baby's face and head about seventy-two hours after those on the thighs. All this evidence points to a mild systemic infection via the upper respiratory or intestinal tracts.

Successful feeding experiments of young guinea pigs rather confirms this idea. A certain per cent. of these animals fed with cultures of the organism in milk developed a pneumonia, the

lungs yielding pure cultures of the organisms fed. One of the adult pigs that suckled the young pigs also died of this type of pneumonia.

The fact that the child when taken from the mother's breast quickly recovered, also points to the conclusion that the mother herself was the infecting source, and that in order to control such infection careful isolation of both mother and child is essential. The mother may harbor the organism in the milk with clinical symptoms that are so little noticeable as to be mistaken for something else, quite insignificant in nature. The freedom from mastitis in this case suggests that the organism lived a commensal existence in this menstruum.

265 (2225)

Water retention in the body.

By W. R. BLOOR and R. G. FREY.

[*From the Laboratories of Biochemistry of the University of California, Berkeley, Calif.*]

This study was undertaken with the purpose of getting some information on the factors influencing water retention in the human body particularly with regard to food substances. Experiments were conducted on medical students of the University, during the late winter which in Berkeley is well adapted to experimentation of this kind since there are long periods of almost constant temperature (about 65° F.) and humidity (75 per cent.) and the laboratories are not heated. Diet was controlled and constant. No liquid was taken after 6 P. M. the night before, breakfast consisting of one egg and a slice of toast with no liquid was eaten at 7:30 A. M. and no luncheon was taken. Urine was voided at 8 A. M. and the first sample collected at 9 A. M. after which one liter of water or the experimental solution was taken and urine samples collected at hourly or half-hourly periods for three or four hours after the diuresis had apparently ceased. Determination of volume, specific gravity, total nitrogen and chlorides were made and the results of sev-

eral experiments of each kind on eight students were briefly as follows:

Water alone. An increased output of urine was noticeable in 30 minutes, reached a maximum in from one to one and one-half hours and was over in four hours. Chloride excretion was irregular but in general fell as the water excretion increased. Two maxima of nitrogen excretion were noticeable—the first about 10 A. M. and the second about 2 P. M. which is noteworthy in view of the fact that no lunch was taken, indicating apparently the influence of habit.

Water and Cane Sugar. No noteworthy effect on the water output was noted until amounts of sugar of over 150 grams were given. With 180 gms. of sugar the increased urine output began and reached approximately the same maximum at the same time as with water alone but decreased much more rapidly reaching the same low point about an hour sooner but showing a *second* moderate maximum at from one to two hours after the first low point. By the end of another hour the output was back to the low (normal) level again, indicating a retention of water by the sugar.

The nitrogen excretion showed the same two maxima as when water alone was given while the chloride did not fall as rapidly as with water alone.

A few experiments were made to determine the amount of salt necessary to cause complete retention of a liter of water for a period of five or six hours. In general about 14 grams of NaCl was found to be necessary.

266 **(2226)**

The effect of germanium dioxide on red cell regeneration in experimental anemia.

By MEYER BODANSKY (by invitation).

[From the Biochemical Laboratory, Department of Physiology and Biochemistry, Cornell University Medical College, Ithaca, N. Y.]

Hammett, Nowrey and Müler,[1] Hammett and Nowrey[2] and Müller and Iszard[3] have reported that germanium dioxide stimulates red cell formation. These authors experimented almost exclusively with normal animals, chiefly rats and guinea pigs. In view of the suggested therapeutic use of germanium dioxide in the treatment of anemia, we investigated the effect of this compound in a number of animals which had been previously made anemic with methyl-phenyl-hydrazine, symmetrical di-iso-propyl-hydrazine 2-2′ azobis-propane and other derivatives of hydrazine.

In the case of dogs, the usual course of recovery from anemia is irregular and varies considerably in different animals. To some extent this is due to temperamental differences in individual dogs and to the inability of certain of these animals to maintain themselves in nitrogen equilibrium. For these reasons, we experienced some difficulty in controlling adequately all of our experiments with germanium.

However, the evidence adduced thus far shows that the erythropoietic action of germanium dioxide, whenever such effect occurs, is transitory. The administration of germanium dioxide over a prolonged period does not appear to alter the degree of red cell regeneration occurring without the use of this compound. Not infrequently, a decrease in the red cell count may follow an injection of germanium dioxide. Where a temporary erythrocythemia was observed in our animals, there was no corresponding increase in the percentage of hemoglobin. The appended chart is illustrative of the effect produced by

1 Hammett, F. S., Nowrey, J. E., and Müller, J. H., *J. Exp. Med.*, 1922, xxxv, 173.

2 Hammett, F. S., and Nowrey, J. E., *Ibid*, 507.

3 Müller, J. H., and Iszard, M., *Am. J. Med. Sci.*, 1922, clxiii, 364.

germanium dioxide, administered over a period of one month to a mildly anemic puppy. In similar experiments in which no germanium dioxide was administered, red cell regeneration occurred as rapidly, and in some instances even more rapidly than in this animal.
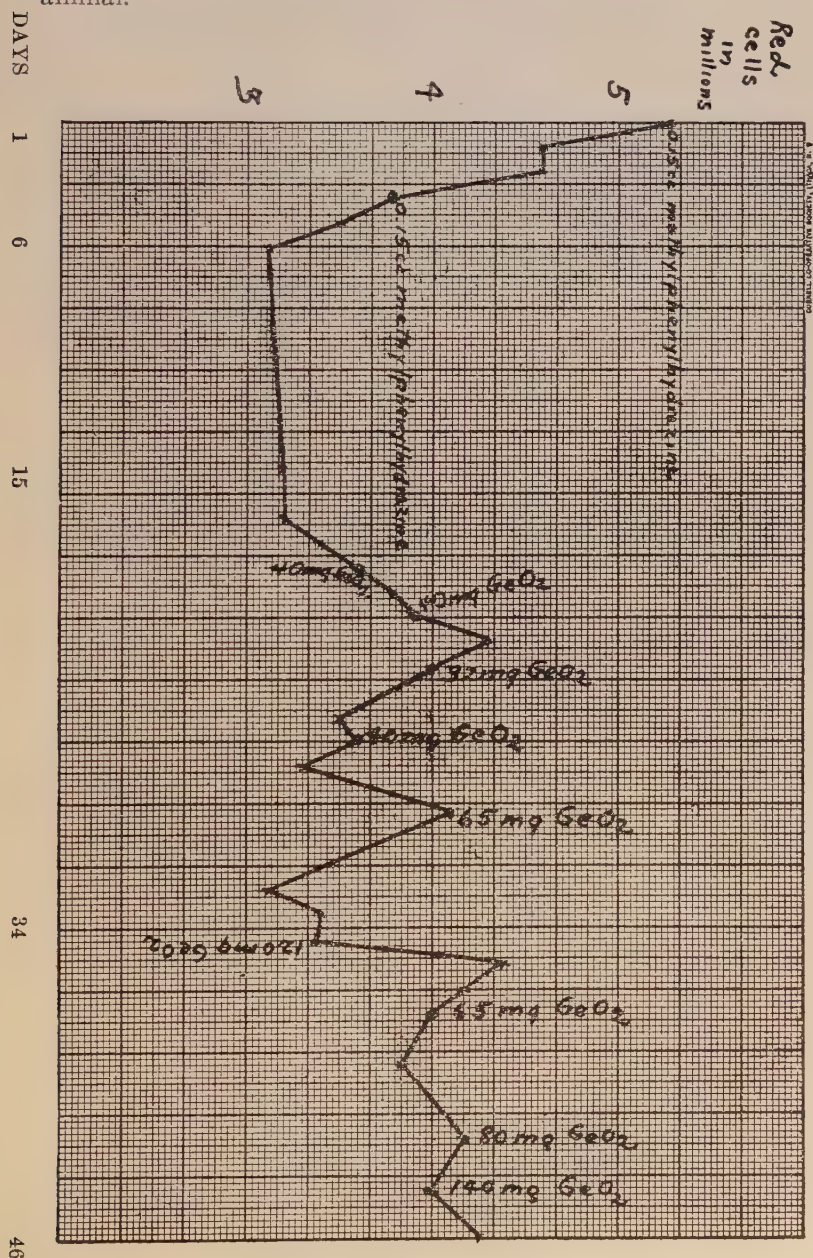
267 (2227)

The effect of germanium dioxide in phenylhydrazine poisoning.

By MEYER BODANSKY (by invitation).

[*From the Biochemical Laboratory, Department of Physiology and Biochemistry, Cornell University Medical College, Ithaca, N. Y.*]

The relative ease with which alterations in blood volume may be produced in rabbits, renders these animals unsuitable for the study of certain phases of experimental anemia. On the other hand, they are more easily maintained in proper nutritive condition and therefore the course of recovery from anemia is more uniform in these animals than in the case of dogs.

In a number of experiments, phenylhydrazine, emulsified in olive oil, was administered subcutaneously in doses sufficient to destroy about two-thirds of the red corpuscles within a period of a week. Germanium dioxide was administered to some of these animals before this interval had elapsed to determine whether any effect was produced in retarding the rate of hemolysis. No such effect was observed. The animals receiving the germanium became as anemic as those not receiving it.

Subsequently, a number of the rabbits received repeated injections of germanium dioxide. The rate of increase of red corpuscles in these animals was compared with that in animals which were not receiving germanium dioxide. It appears from our results, that germanium dioxide in moderate amounts does not augment the rate of red cell regeneration in rabbits recovering from phenylhydrazine poisoning.

The germanium dioxide used in this work was supplied to us by Professor L. M. Dennis of Cornell University, to whom we are indebted.

268 (2228)

The effect of fasting and of vitamin B deprivation on the chemical composition of rats' blood.

By H. A. MATTILL.

[*From the Department of Physiology, University of Rochester, Rochester, New York.*]

The recent publication of data on the blood of pigeons in polyneuritis and stavation[1] suggested the desirability of publishing the data obtained on rats under similar conditions. The preliminary report[2] on the influence of fasting and of vitamin B starvation has been amplified and data have also been obtained on fasting rats to whom water (10 to 21 c.c. daily) was administered by stomach tube during the last days of the fast. Total solids were determined by an adaptation of Peters' method[3] and the other constituents by the methods of Folin and Wu.[4]

From the summary given in the table it is apparent that the non-protein nitrogen of the blood of fasting rats is 30-40 per cent higher than that of normal animals, the increase being practically all in the urea fraction. Total dry matter, creatinine and creatine are slightly increased. Fasting rats that are given water *per os* during the last few days of their fast show normal values for non-protein nitrogen and solids. The blood of rats deprived of vitamin B also gives figures that are normal except that creatinine is at the fasting level and creatine slightly higher than the fasting figure; these differences have little if any significance in the present state of uncertainty with regard to blood creatine and creatinine determinations. Deprivation of vitamin B in contrast to fasting is not accompanied by an accumulation of nitrogenous end-products in the blood; whatever the ultimate cause of this increase may be in fasting, it is prevented by administration of water. The commonly observed disinclination of fasting animals to drink water was first shown by Bang[5] to

1 Palmer, L. S., and Hoffman, C. T., PROC. SOC. EXP. BIOL. AND MED., 1922, xx, 118.

2 Mattill, H. A., *Science*, 1921, liv, 176.

3 Peters, A. W., *J. Biol. Chem.*, 1919, xxxix, 285.

4 Folin, O., and Wu, H., *J. Biol. Chem.*, 1919, xxxviii, 91.

5 Bang, I., *Biochem. Z.*, 1916, lxxii, 119.

be responsible for the increased non-protein nitrogen and urea which he found in the blood of fasting rabbits. A similar condition seems to obtain in fasting rats leading to a relative concentration of the blood as contrasted with its dilution in the fasting pigeon. In this connection the results of Arima[1] on human blood are of interest in showing that the non-protein nitrogen in various forms of beri-beri is 32 to 67 mg. per 100 c.c.

AVERAGE ANALYSES OF RATS' BLOOD

Condition	Number of animals	Per cent. loss in weight	Total solids per cent.	Total non-protein nitrogen	Urea nitrogen	Preformed Creatinine	Total Creatinine	Creatine
				milligrams per 100 c.c.				
Normal	11		21.1	41	21	1.1	2.3	1.2
Without B .	17	20-44	21.5	39	19	1.3	3.0	1.7
Fasting	10	22-37	23.2	67	40	1.3	2.8	1.5
Fasting with forced water intake	5	28-37	21.2	39	18			

269 (2229)

Antagonistic effects of insulin and thyroxin.

By AARON BODANSKY (by invitation).

[From the Department of Physiology and Biochemistry, Cornell University Medical College, Ithaca, N. Y.]

Continued subcutaneous administration of thyroxin is known to produce hyperglycemia. In normal sheep the blood-sugar is raised from about 70 mg. to over 80 mg.

A single subcutaneous administration of thyroxin has only a slight effect on the blood sugar.

A single *subcutaneous* administration of insulin (10 units) produced no demonstrable effect within two hours after injection.

Intravenous administration of varying amounts of insulin (5

[1] Arima, E., 1916, abstracted in *Chem. Abstr.*, 1918, xii, 64.

to 15 units) produced prompt hypoglycemia in normal sheep, the sugar decreasing from about 70 to about 40 mgs. per 100 c.c. of blood within about 30 minutes. (No unfavorable symptoms were observed). The drop is regular, and when plotted is repre-

sented by a practically straight line. The slope of this portion of the curve is constant for the same sheep upon administration of varying amounts of insulin.
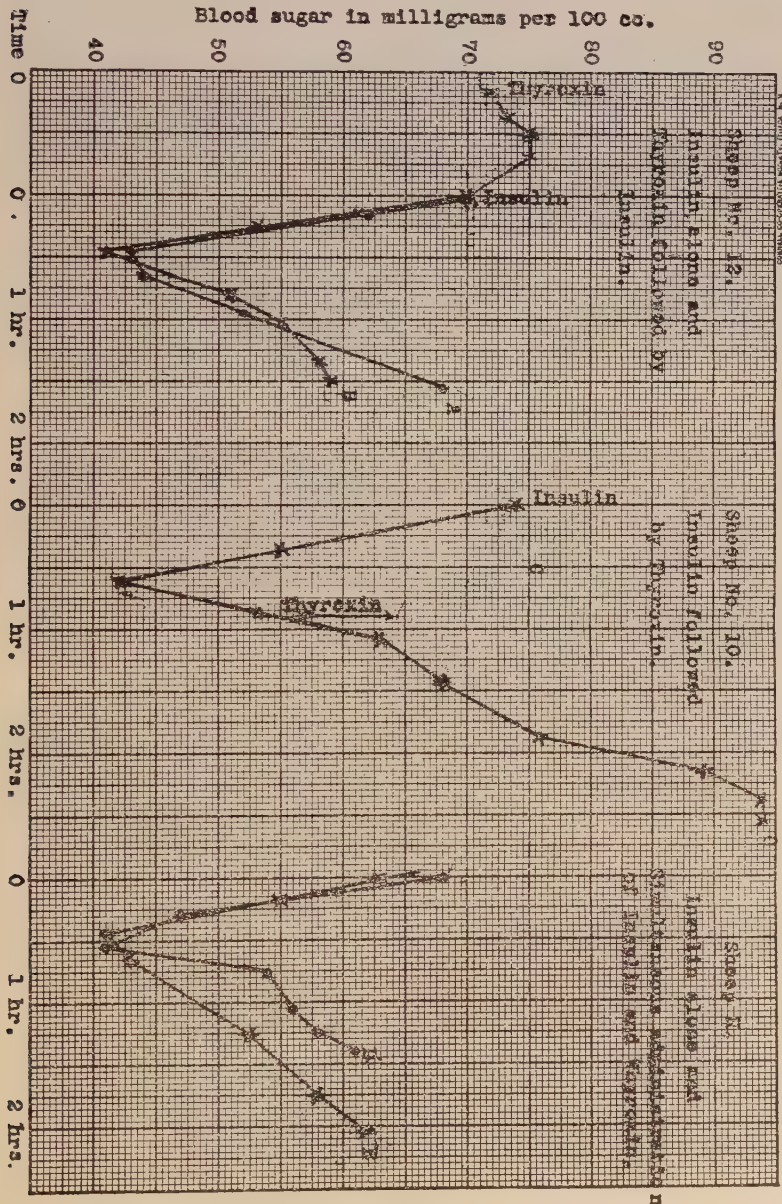
The portion of the curve representing recovery from hypoglycemia shows characteristic differences: When 5 units of insulin were administered, the recovery to normal sugar values began immediately after the low values had been reached. With 10 to 15 units the minimum sugar values obtained were only slightly lower than for the smaller doses, although analyses were taken at sufficiently close intervals (10 to 20 minutes) to enable a fair approximation to the minimum. The larger doses, however, showed an extensive flat portion in the curves before recovery began. The results with five units were reproducible with the same sheep and similar in different sheep.

Five units were therefore selected as the standard dose of insulin to be tested against varying amounts of thyroxin under different conditions. Administered alone, the effects produced are represented by curves A and D. Curve B shows the effect of a preliminary administration of thyroxin (1 mg. intravenously). Curve C shows the effect of a similar injection of thyroxin after the recovery from insulin had begun. The simultaneous administration of thyroxin (1.5 mg.) and insulin produced a typical divergence of the ascending arm of curve E from that of curve D (insulin alone). With a smaller dose of thyroxin (0.75 mg.) in another sheep a similar effect was obtained, though not quite as pronounced as in Sheep H.

These results may be explained tentatively on the following assumptions: Insulin causes an accumulation of glycogen in the liver, simulating the condition in a well fed animal; subsequent administration of thyroxin produces the marked hyperglycemia due to increased glycogenolysis, shown in Curve C. A preliminary administration of thyroxin increases sugar metabolism, depletes the glycogen store, thus simulating the condition in a poorly nourished animal, when recovery from a subsequent administration of insulin occurs, the normal blood-sugar levels are not reached therefore within the usual time.

This work was carried out under a grant from the Heckscher Research Foundation to Professor Sutherland Simpson. Further work is in progress on the relations of thyroxin and insulin, and their possible application to the assay of insulin.

270 **(2230)**

A study of phloridzin and its derivatives. Part I.

By ALBERT A. EPSTEIN and EUGENIE HIRSCHBERG-MAECHLING.

[*From the Laboratory of Physiological Chemistry of Mt. Sinai Hospital, New York City.*]

The present work constitutes part of a study on the pharmacology of phloridzin and its derivatives. It is based on some earlier observations[1] concerning the influence of phloridzin on the formation of glycogen in the liver under certain experimental conditions. The results of the experiments referred to seemed to indicate that phloridzin possesses a two-fold action: namely, that of causing glycosuria, and that of stimulating the formation of glycogen in the liver. These experiments showed further that animals which had been rendered diabetic by pancreatectomy, and in which the glycosuria was interfered with by removal of the kidneys, the blood-sugar content was generally lower in those which had received phloridzin, than in those which had not received it.

It therefore seemed reasonable to suppose that if a means could be found by which phloridzin could be deprived of its glycosuric action, its effect on the formation of glycogen and the reduction of the blood-sugar would be made manifest.

Notwithstanding the importance of phloridzin in the study of certain problems of metabolism, little information is available concerning its derivatives.

In view of the object of our investigation the work is comprised of two phases: first, an examination of the pharmacological effects of known derivatives of phloridzin and second, the preparation and study of new compounds of this substance.

The purpose of the present communication is to state our problem and to record the new compounds which have been prepared and identified. The following is a list of the substances:

(1.) Tetrabenzoylphloridzin: This was obtained by the action of benzoyl chloride on phloridzin in pyridin. The sub-

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1 Epstein, A. A., and Baehr, G., *Jour. Biol. Chem.*, 1916, xxiv, 1.

stance is white, and of a starchy character. It melts at 94.5°-96° C. It is readily soluble in alcohol, acetone, pyridin, slightly soluble in ether, and is insoluble in ligroin, alkalies or water.

(2.) Tetra-para-nitro-benzoylphloridzin: This was obtained in a manner analogous to that of the preceding substance, by the reaction of para-nitro-benzoyl chloride on phloridzin in pyridin. This substance is amorphous in character, and of a greenish yellow color. Melting point is 122° C. It is readily soluble in pyridine, less so in boiling alcohol and acetone; slightly soluble in ether, and insoluble in ligroin alkalies and water.

(3.) Pentapalmitylphloridizin: Was prepared from phloridzin and palmitylchloride in pyridin solution. It forms a slightly yellowish amorphous product, melting sharply at 51.5° C; easily soluble in chloroform, alcohol, ether, acetone, pyridin and ligroin.

(4.) Sodium phloridzin-tris-azobenzene: Was prepared in an alkaline medium by the reaction of diazobenzene sulfate with phloridzin. The substance forms a bright red amorphous mass, which is readily soluble in ethyl alcohol, methyl alcohol, acetic acid, acetone and ether; it is insoluble in ligroin and water. Dilute alkalies dissolve it with the formation of a dark red color. Concentrated sulfuric acid dissolves it, giving the solution a deep red color. This substance turns dark at 190° C. and at 405° C. it decomposes.

Three other new compounds have been prepared; their chemical constitution, however, has not as yet been definite established: (1) phloridzin-sulfanildiazotate, (2) phloridzin-diazoorthotoluene, (3) phloridzin-diazoparatoluene.

A complete report of the methods used in the preparation of the above substances, as well as their physical properties and chemical constitution will be presented in another place. The pharmacological action of these compounds will be reported later.

We wish to express our thanks to Dr. A. Elek of the Rockefeller Institute for the combusion analyses of these new compounds.

271 (2231)

The nitrogen content of the pneumococcus: A preliminary report.

By FRANZ LEINEWEBER, RUTH KAUTSKY, and L. W. FAMULENER.

[*From the Pathological Laboratory, St. Luke's Hospital, New York City.*]

A study of the nitrogen content of various bacterial groups and subgroups was undertaken by the writers some months ago. The object was to determine, if possible, the relationship of the nitrogen content which might occur between members of a single group, and also that between the subgroups of a species where such are known to exist. While progress has been made in this study, much remains to be done, and we are continuing the investigation. A preliminary report of the results of our analyses made upon the pneumococcus, including the four serological subgroups, may prove to be of interest to others. We therefore submit some of the data, although the study is not completed.

Recognizing the many known factors which may enter and alter the results in a study of the protein content of bacteria, we have attempted to control these factors in so far as possible by keeping them constant throughout. In this way, to a certain degree, a comparison is possible between the derived chemical data.

The organisms used in this study were for the most part recovered from sputum, blood, etc., of patients in St. Luke's Hospital, although we are indebted to Dr. Avery, of the Rockefeller Institute, and to Dr. Wadsworth, of the New York State Health Department Laboratories, for a few strains of the Type II. In each instance the identity of the organism was established by its characteristic morphology, staining properties, bile solubility, and reaction to specific immune serum. Ten strains of each of the four chief groups were cultured in flasks containing about 75 c.c. of a beef infusion broth containing one per cent. of dextrose, and having an average reaction of P_H 7.4 to 7.6. Usually sufficient growth for the purpose of the study was produced within 24 hours, when incubated at 36° or 37° C. The sedimented organisms were removed from the bottom of the flask by means of a Pasteur pipette, placed in a Hopkins vaccine tube, centrifuged, and the supernatant fluid removed from above the

packed sedimented bacteria. Physiological salt solution (0.85 per cent.) was added to the sediment in the proportion of 150 to 200 volumes of salt solution to one volume of the packed organism. The organisms were thoroughly washed in this fluid, and then re-centrifuged at a high speed for thirty minutes, until the sediment failed to pack further. The supernatant salt solution was removed, and if necessary, the upper zone of the packed bacterial column in the small calibrated portion of the vaccine tube. Sufficient physiological salt solution was added to make a 1 per cent. suspension by volume. The suspension was subjected to heat in the water bath for one hour at 60° C. to kill the organism. Tests were made for sterility. No preservative was added.

The nitrogen determination was made upon aliquot parts of the killed bacterial suspension.

The total nitrogen content of the bacteria was determined by the micro-chemical method of Folin and Farmer. For this purpose, 1 c.c. samples of the homogeneous suspension of the organism were used for each estimation. The tests were made in duplicate, and if the results did not closely agree in each instance, the tests were repeated. It is noteworthy that the results of such tests showed a close agreement. The total solids were determined by placing 5 c.c. of the homogeneous suspension of the organism in a small tared porcelain crucible, then removing the water content in so far as possible in an electric oven kept at a temperature of 65° to 70° C. Finally the crucibles were placed in desiccator containing phosphorus pentoxide. A vacuum was produced, then it was placed in the electric oven (65° to 70° C.) until the crucible with its contents came to a constant weight. From the total solid content of the dried solids of each specimen, the added sodium chloride (physiological salt solution used in preparation) content was deducted, leaving the amount of total bacterial solids. The percentage of bacterial nitrogen was estimated upon this basis.

In general, the nitrogen contents of the individual strains (ten) in each of the four serological groups of pneumococcus were approximately of the same amount, although an occasional exception was observed, either a slightly higher or a lower value than the general mean, which did not alter the average to any extent. This occurred in the Group I series of determinations

in three instances. New bacterial preparations were made from each strain and the nitrogen contents were determined. The results of the second series showed but slight variation from the first. We are inclined to the view that either a relatively high or a low nitrogen content of certain strains may be an inherent quality, slightly deviating in this respect from the average strain, when grown under parallel conditions. Group III strains seemed to fall into two subgroups when based upon their nitrogen content; one (a) subgroup (four strains) showed an approximately lower percentage of nitrogen than the second (b) subgroup (six strains), which showed a nitrogen content similar to the three other serological groups. A number of possible factors which might cause this variation have been considered, but as yet, no one has proved to be the direct cause. Averages of the results taken from ten strains analyzed in each of the serological groups gave the following total nitrogen content:

Group I, 9.4 per cent.; Group II, 10 per cent.; Group III, 8 per cent. (subgroup "A" gave 7.43 per cent.; subgroup "B" gave 9.3 per cent.); Group IV, 8.8 per cent. As is apparent, the total nitrogen content of the four general serological groups does not greatly deviate from a mean average of 9 per cent., a mean which is reduced by using a general average of 8 per cent. for Group III. It must be emphasized that these values are not considered fixed, since a parallel series might slightly alter the percentage results.

272 (2232)

The transmission of the virus of herpes febrilis along sensory nerves with resulting unilateral lesions in the central nervous system in the rabbit.

By ERNEST W. GOODPASTURE and OSCAR TEAGUE.

[*From the Williams H. Singer Memorial Research Laboratory Allegheny General Hospital, Pittsburgh, Pennsylvania.*]

Rabbits inoculated upon the cornea of the right eye with our virus of *Herpes febrilis* show constantly on about the fourth or fifth day a turning of the head toward the right side; during the succeeding days the neck is twisted strongly toward the

right and held rigidly in that position. This picture was described by Doerr and Schnabel and later by Levaditi and was attributed by Doerr and Schnabel to an encephalitis, the virus probably passing from the cornea to the brain by way of the blood stream. Levaditi, on the other hand, claimed to have proved that the virus passed from the cornea along the optic nerve to the brain. Our observations make it evident to us that this symptom is due to a lesion on the right side of the pons and medulla along the distribution of sensory fibers of the fifth cranial nerve, which involves the roots of the spinal accessory nerve supplying muscles of the neck on the same side, and that this lesion is produced by the virus of herpes entering the brain from the cornea by way of the sensory portion of the fifth cranial nerve. No other lesion has been observed in the brain which might be attributed to passage of virus from the eye along any other nerve, and the unilateral situation of the lesion precludes its production by transmission of the virus through the blood stream. At autopsy on a rabbit inoculated into the right eye killed after the appearance of a turning of the head to the right, one frequently finds macroscopic hemorrhages situated at the point of entrance of the fifth cranial nerve and along the side of the pons and medulla corresponding approximately to the distribution of sensory fibers and nucleus of this nerve on the right. Microscopically an acute inflammation is found with degeneration, necrosis and infiltration with polynuclear and mononuclear cells limited to the right side of pons and medulla corresponding to the gross lesions, sometimes descending superficially on the same side along the dorso-lateral portion of the cord involving the root of the first cervical nerve. This lesion has not been observed to extend upward from the entrance of the fifth cranial nerve.

In such a lesion, cells of glial and ganglion types are found to contain intra-nuclear inclusion-bodies similar to those described by Lipschütz in the cornea of rabbits inoculated with virus of herpes febrilis, and which we regard as pathognomonic of herpetic lesions in general.

The following additional experiments may be cited as confirming the passage of this virus along sensory nerves:

(1). A rabbit inoculated into the skin of the right hind leg developed on the 10th day impairment in the use of this leg, progressing during the next few days to practically complete

disability of this extremity. On the 11th day impairment in function of the left leg followed. At autopsy the lumbar region of the cord showed gross hemorrhages limited to the right dorsal surface of the lumbar portion of the cord along the line of entrance of dorsal root fibers. Microscopic sections showed lesions like those found in the pons and medulla including the presence of intra-nuclear inclusion-bodies, involving the right dorsal and a portion of the lateral area of the cord, with an extension along the posterior commissural fibers to the median portion of the left dorsal cord.

Following inoculation of the skin with virus of herpes, Levaditi noted paralysis of the posterior extremities in a rabbit but clearly states that no lesions were found in the cord.

(2.) A rabbit inoculated into the peritoneum of the abdominal wall to the left of the median line developed on the 15th day a lateral curvature of the spine with convexity toward the left side. Microscopic sections of the dorsal cord showed a degeneration of sensory fibers situated dorsally and median to the entrance of dorsal root fibers limited to the left side.

(3.) A rabbit inoculated into the left adrenal developed on the fifth day impairment in function of both hind legs with apparent paralysis of abdominal muscles.

Sections at different levels of the cord showed in the lower dorsal region, acute lesions, as described in pons and medulla, involving the left side along the entrance of sensory root fibers.

In this case we assume the virus passed along the sympathetic fibers from the adrenal, entering the cord through the dorsal root.

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Lundsgaard, Christen.
    2030.   [with **Knud Schierbeck.**]   Studies on lung volume. IV. Investigations on admixture of air in the lungs with other air.
    2031.   [with **Knud Schierbeck.**]   Studies on lung volume.

Mann, Hubert.
    2174. See Openheimer, B. S.
Mann, F. C.
    2214 [with Thomas B. Magath.] The effect of insulin on the blood sugar following total and partial removal of the liver.
Manwaring, W. H.
    1982. [with Walter H. Boyd and William O. French.] Reactions of the capillary endothelium in peptone shock.
    1983. [with Walter H. Boyd.] Study of bacterial toxins by means of the isolated mammalian heart.
    2046. [with R. C. Chilcote, W. S. Clark and R. E. Monaco.] The dominant reacting tissues in anaphylactic peptone and histamine shock.
    2047. [with R. E. Monaco and H. D. Marino.] Histamine reactions in isolated canine tissues.
    2048. [with R. E. Chilcote and Selling Brill.] The hepatic mechanical factor in canine anaphylactic shock.
    2090. [with R. C. Chilcote and V. M. Hosepian.] The endothelial factor in anaphylaxis.
    2091. [with R. C. Chilcote and V. M. Hosepian.] Types of canine anaphylaxis.
    2095. [with R. C. Chilcote and V. M. Hosepian.] Anaphylactic reactions in isolated canine organs.
Marino, H. D.
    2047. See Manwaring, W. H.
Marsh, M. Elizabeth.
    2020. Energy metabolism of premature infants.
Mattill, H. A.
    2161. See Piper, H. A.
    2165. [with J. S. Carman.] The degeneration of the testis of rats on a milk diet.
    2228. The effect of fasting and of vitamin B deprivation on the chemical composition of rat's blood.
Matzner, M.
    1996. See Hess, A. F.
Maver, James W.
    2122. An effect of X-rays on crossingover in Drosophila.
Maynard, L. A.
    2223. [with S. A. Goldberg, K. V. Williams and O. B. Cristie.] Further studies on "posterior paralysis" in swine.

McCann, W. S.
>2199.   See **Lyman, R. S.**

McClendon, J. F.
>2009.   [with **O. S. Rask.**]   The determination of iodine in large samples of foodstuffs.
>2098.   [with **Agnes Williams.**]   Experimental goitre and iodine in natural waters in relation to distribution of goitre.
>2099.   The presence of anti-ophthalmic vitamin and the absence of anti-rachitic vitamin in dried spinach.
>2131.   [with **Cecilia Shuck.**]   The determination of iodin in iodin metabolism.

McLean, Franklin C.
>2066.   See **Van Slyke, D. D.**

McMaster, P. D.
>2023.   See **Rous, Peyton.**
>2114.   See **Rous, Peyton.**

Meleney, Henry E.
>2065.   [with **E. C. Faust.**]   The intermediate host of schistosoma japonicum in China.
>2153.   [with **E. C. Faust.**]   The route of migration of schistosoma japonicum in the body of its final host.

Mellon, Ralph R.
>2052.   Observations on the origin of biotypes (microbic dissociation) in pure lines of bacteria.
>2053.   Observations on the relation of bacterial giant coccoids to zygospore formation.
>2224.   [with **Willard S. Hastings** and **Dorothy W. Caldwell.**]   Observations on the epidemiology of pemphigus neonatorum.

Mendel, L. B.
>2074.   See **Deuel, H. J., Jr.**
>2186.   See **Osborne, T. B.**

Metz, C. W.
>2110.   A note on the effects of temperature on the mutant character "bent" in drosophila virilis and drosophila melanogaster.

Minoura, Tadachiki.
>2188.   See **Riddle, Oscar.**

Mitchell, O. H. W.
>2159.   Friedländer bacillus bacteremia.

Moise, T. S.
    2180.   See Smith, Arthur H.
Monaco, R. E.
    2046.   See Manwaring, W. H.
    2047.   See Manwaring, W. H.
Montank, Irwin A.
    2130.   See Larson, W. P.
    2171.   See Larson W. P.
Muldoon, J. A.
    1979.   [with G. J. Shipley and C. P. Sherwin,]   Is cystin synthesized in the animal body?
Murlin, John R.
    1989.   See Clough, Harry D.
    1990.   See Gibbs, C. B. F.
    1991.   See Sutter, C. Clyde.
    1992.   Properties and methods of preparation of the anti-diabetic substance (glucopyron) generated by the pancreas.
    2057.   See Gibbs,, C. B. F.
    2161.   See Piper, H. A.
    2163.   See Clough, H. D.
    2216.   See Allen, R. S.
Murray, T. J.
    2108.   Food accessory substances and the nitrite bacteria.
Myers, V. C.
    2135.   [with H. W. Schmitz and Leila E. Booher.] A micro colorimetric method of estimating the hydrogen ion concentration of the blood.

Nelson, Edmund.
    2130.   See Larson, W. P.
Newcomb, E. L.
    2097.   Observations on the assay and factors influencing the quality of digitalis.
Nicholls, Elizabeth.
    2199.   See Lyman, R. S.
Oliver, Jean.
    1984.   The relative therapeutic efficiency of arsphenamine and gelatine-arsphenamine.
Oppenheimer, B. S.
    2174.   [with Hubert Mann.] An electrocardiographic sign in pericardial effusions.

Osborne, T. B.
 2186. [with **L. B. Mendel, E. A. Park** and **D. Darrow.**] Kidney hypertrophy produced by diets unusually rich in protein.
Outhouse, Julia.
 2185. See **Dutcher, R. Adams.**

Packard, Charles.
 2069. The susceptibility of cells to radium radiation.
Palmer, L. S.
 2017. [with **Clara T. Hoffman.**] Biochemical properties of the blood of pigeons in polyneuritis and starvation.
 2210. [with **Cornelia Kennedy.**] Growth and reproduction of rats on whole milk as the sole diet.
Pappenheimer, A. M.
 1964. See **Hess, A. F.**
Park, E. A.
 2000. See **Powers, G. F.**
 2186. See **Osborne, T. B.**
Parker, Frederic, Jr.
 1968. [with **Julia T. Parker.**] Observations of fluctuations of virulence of B. influenzae.
Parker, Julia T.
 1968. See **Parker, Frederic, Jr.**
Parsons, T. R.
 2076, See **Lamer, Victor K.**
Pearce, Louise.
 2195. See **Brown, Wade H.**
 2196. See **Brown, Wade H.**
Perlzweig, W. A.
 2142. [with **G. I. Steffen.**] On the nature of pneumococcus antigen.
Paton, Julia B.
 2168. See **Funk, Casimir.**
Peterson, Emelia.
 2207. [with **Ivan C. Hall.**] The isolation of bacillus histolyticus from soil in California.
Pilz, G. F.
 2041. [with **W. J. Crozier.**] Action of drugs upon the central nervous system of insects.

Piper, H. A.
    2161.   [with H. A. Mattill and John R. Murlin.]   Further observations on the chemical and physical properties of insulin.
    2162.   See Kimball, C. P.
    2216.   See Allen, R. S.
Pinkerton, Henry.
    2089.   See Wolbach, S. B.
Pohlman, A. G.
    2029.   [with F. W. Kranz.]   On the effect of certain drugs, notably quinine, on the acuity of hearing.
    2150.   [with F. W. Kranz.]   A new auditory test apparatus.
Porter, Eugene L.
    2197.   [with Victor W. Hart.]   Reflex contractions of an all-or-none character.
Powers, G. F.
    2000.   [with E. A. Park and Nina Simmonds.]   The influence of light and darkness upon the development of xerophthalmia in the rat.
Pucher, G. W.
    1987.   See Cori, Karl F.
    2217.   [with K. F. Cori and B. D. Bowen.]   The fate of iletin in the animal body.
    2218.   See Cori, K. F.
    2219.   See Cori, K. F.

Rabinowitz, M.
    2070.   See Scott, F. H.
Rask, O. S.
    2009.   See McClendon, J. F.
Rasmussen, A. T.
    2011.   Experimental demonstration of the entire course of four descending tracts by a single alcoholic injection in the mid-brain of the cat.
    2015.   The occurrence of multilocular fat cells in the perirenal back of man.
Read, Bernard E.
    2152.   [with Carl F. Schmidt.]   The pharmacology of Tang Kuei.

Riddle, Oscar.

2077. Resistance of pigeons to the lethal action of iletin (insulin) with observed effects on reproduction.

2078. See Honeywell, H. E.

2188. Effects of repeated transplantation of whole suprarenals into young doves.

Robertson, O. H.

1973. [with Peyton Rous.] Lasting individual differences in the resistance of normal bloods to shaking.

2068. [with Richard H. P. Sia.] A method for demonstrating growth-inhibitory and bactericidal action on the pneumococcus of a normal serum-leucocyte mixture.

Rogoff, J. M.

1972. See Stewart, G. N.

1975. See Coombs, Helen C.

2124. See Stewart, G. N.

2125. See Stewart, G. N.

Roth, George B.

1976. Barium-epinephrin antagonism on the excised surviving intestine.

Rous, Peyton.

1973. See Robertson, Oswald H.

2023. [with P. D. McMaster and G. O. Broun.] The experimental production of gall-stones in dogs, in the absence of infection, stasis and gall-bladder influences upon the bile.

2114. [with P. D. McMaster and D. R. Drury.] The genesis of gall-stones in the dog.

Rose, A. R.

1997. See Denise, Sister Mary.

2134. See Shiple, G. J.

Rosenthal, Sanford M.

2002. A new method of testing liver function with phenoltetrchlorphthalein.

Rosenthal, Nathan.

2024. [with George Baehr.] The paradoxyal shortening of blood coagulation after intravenous administration of sodium citrate.

Rupp, A.

2070. See Scott, F. H.

Salant, William.
    2149. [with **Robert L. Johnston.**] The action of salicylate on the isolated heart.

Salvesen, Harald.
    2061. Studies on the physiology of the parathyroids.

Sansby, J. Martin.
    2013. See **Siperstein, David M.**

Scammon, R. E.
    2010. The height-weight index of the newborn infant.
    2016. [with **Talbot L. Dunn.**] Empirical formulae for the post-natal growth of the human brain and its major divisions.
    2132. [with **L. A. Calkins.**] Simple empirical formulae for expressing the lineal growth of the human fetus.

Schierbeck, Knud.
    2030. See **Lundsgaard, Christen.**
    2031. See **Lundsgaard, Christen.**
    2032. See **Lundsgaard, Christen.**
    2033. See **Lundsgaard, Christen.**
    2034. See **Lundsgaard, Christen.**
    2035. See **Lundsgaard, Christen.**

Schlesinger, M. J.
    2089. See **Wolbach, S. B.**

Schloss, Oscar M.
    1962. [with **Arthur F. Anderson.**] Allergy to cow's milk in infants with severe malnutrition.

Schmitz, H. W.
    2135. See **Myers, V. C.**

Schmidt, Carl F.
    2152. See **Read, B. E.**

Scott, F. H.
    2018. See **Bieter, R. A.**
    2070. [with **M. Rabinowitz** and **A. Rupp.**] The effect of increase of bloodpressure on the concentration of colloidal dyes in the plasma.

Schuck, Cecelia.
    2099. See **McClendon, J. F.**

Seymour-Jones, F. L.
    2175. See **Thomas, Arthur W.**

Shaughnessy, H. J.
    2171. See **Falk. I. S.**

Sheets, Olive.

    1999.   [with **Casimir Funk.**]   The effect of ultra violet rays on rats, deprived of vitamin A in their diet.

Shell, G. S.

    2205.   See **Clark, Guy C.**

Sherburne, C. C.

    2079.   See **Kinsella, Ralph A.**

Sherman, H. S.

    2059.   [with **M. M. Kramer.**] Experiments on vitamin A.

Sherwin, C. P.

    1979.   See **Muldoon, J. A.**

    2022.   See **Bullowa, Jesse G. M.**

    2134.   See **Shiple, G. J.**

Shiple, G. J.

    1979.   See **Muldoon, J. A.**

    2134.   [with **A. R. Rose** and **C. P. Sherwin.**] Cystin metabolism.

Shohl, A. T.

    2028.   The quantitative determination of the alkali retention in growth.

Short, J. J.

    1978.   See **Kast, Ludwig.**

Sia, Richard H. P.

    2068.   See **Robertson, O. H.**

Simmonds, Nina.

    2000.   See **Powers, G. F.**

Simpson, Sutherland.

    2055.   See **Bodansky, A.**

    2056.   See **Liddell, H. S.**

    2160.   Thyroparathyroidectomy in the rabbit.

Siperstein, David M.

    2013.   [with **J. Martin Sansby.**] The intraperitoneal transfusion of citrated blood.

Smith, Irene B.

    1981.   See **Smith, Philip E.**

Smith, A. H.

    1977.   [with **W. L. Kulp.**] The effect of change in type of intestinal bacteria on urinary indican and phenols.

    2180.   [with **T. S. Moise.**] Diet and tissue regeneration.

Smith, P. E.
      1981.   [with Irene B. Smith.]   Retardation of metamorphosis in the Colorado axolotl by the intraperitoneal injection of fresh bovine hypophyseal anterior lobe substance.

Somerfield, H. A.
      2204.   See De Eds, F.

Speidel, C. C.
      2144.   See Jordan, H. E.

Spencer, Hope.
      2123.   See Woodruff, L. L.

Spiridonovitch, R.
      2136.   Some studies on the vital stain of blood cells.

Starkey, Robert L.
      1963.   See Waksman, S. A.

Steffen, G. I.
      2142.   See Perlzweig, W. A.

Stenstrom, Wilhelm.
      1988.   Possibility of increasing the value at depth of radiation from radium externally applied.

Stewart, G. N.
      1972.   [with J. M. Rogoff.]   The supposed relation of the adrenals to reflex volume changes in denervated limb.
      2124.   [with J. M. Rogoff.]   The effect of iletin (insulin) on the blood sugar content in adrenalectomized animals.
      2125.   [with J. M. Rogoff.]   The influence of iletin (insulin) on morphine hyperglycemia.

Stillman, Edgar.
      2115.   See Linder, G. C.

Stokes, A. M.
      1989.   See Clough, Harry D.

Stomberg, C. W.
      2100.   See Green, R. G.

Stone, Neil C.
      1989.   See Clough, Harry D.
      1990.   See Gibbs, C. B. F.

Sumner, James B.
      2007.   [with V. A. Graham.]   Dinitrosalicylic acid as a reagent for blood sugar.
      2184.   Concerning the detection of pentose, formaldehyde and methyl alcohol.

Sutter, C. Clyde.

    1991. [with **John R. Murlin.**] Three month's study of the influence of the antidiabetic substance on a case of severe diabetes.

    2164. See **Gibbs, C. B. F.**

Swanson, W. W.

    2156. See **Hirschfelder, A. D.**

Swingle, Wilbur W.

    2146. See **Woodruff, L. L.**

Tainter, M. L.

    2203. [with **P. J. Hanzlik.**] The mechanism of edema production by paraphenylenediamin.

Teagarden, E. J., Jr.

    2147. See **Macht, David I.**

Teague, Oscar.

    2155. See **Goodpasture, E. W.**

    2232. See **Goodpasture, E. W.**

Ten Broeck, Carl.

    2154. [with **J. H. Bauer.**] The transmission of tetanus antitoxin through the placenta.

Thomas, Arthur W.

    2175. [with **F. L. Seymour-Jones.**] The hydrolysis of collagen by trypsin.

Thomas, J. E.

    2081. See **Kuntz, Albert.**

Thro, William C.

    2113. [with **Marie Ehn.**] Calcium in the blood.

Tolstoi, E.

    2139. See **Hess, Alfred F.**

Uhlenhuth, Edouard.

    2202. The elaboration and release of the colloid of the thyroid.

Updegraff, Helen.

    2037. See **Lewis, Howard B.**

Vanderlingen, J. S.

    2187. [with **D. I. Macht.**] A contribution to the biophysics of intestinal absorption.

Van Sant, Helen M.

    2067. See **Young, Charles W.**

Van Slyke, D. D.
    2060.   [with **Hsien Wu** and **Franklin C. McLean.**]
Factors controlling the electrolyte and water distribution in the
blood.
    2115.   See **Linder, G. C.**
    2116.   See **Linder G. C.**

Waksman, Selman A.
    1963.   [with **Robert L. Starkey.**]   Carbon assimilation
and respiration of autotrophic bacteria.
Weinstock, M.
    1964.   See **Hess, Alfred F.**
    2139.   See **Hess, Alfred F.**
Weiss, Soma.
    2060.   Some modifications of Emil Fischer's microcolori-
meter.
    2111.   [with **Robert A. Hatcher.**]   Localization of the
vomiting center.
Willaman, J. J.
    2012.   See **Anderson, Arthur K.**
Williams, Agnes.
    2098.   See **McClendon, J. F.**
Williams, K. V.
    2223.   See **Maynard, L. A.**
Wilson, D. C.
    2049.   Vital capacity determinations in persons with nor-
mal heart and lungs above forty years of age.
Winslow, C.-E. A.
    2172.   [with **I. S. Falk** and **M. F. Caulfield.**]   The in-
fluence of certain electrolytes upon the electrical charge of
bacteria.
Wolbach, S. B.
    2089.   [with **Henry Pinkerton** and **M. J. Schlesinger.**]
The cultivation of the organisms of Rocky Mountain spotted
fever and typhus in tissue cultures.
Woodruff, L. L.
    2123.   [with **Hope Spencer.**]   Paramecium polycaryum
sp. nov.
    2146.   [with **W. W. Swingle.**]   The effect of thyroid
products on paramecium.
Wu, Hsien.
    2066.   See **Van Slyke, D. D.**

Young, Charles W.

  2067. [with **Helen M. Van Sant.**] Leishmania donovani in the peripheral blood.

Zucker, Theodore F.

  1967. [with **W. C. Johnson** and **Marion Barnett.**] The acid-base ratio of the diet in rickets production.

  2025. [with **Margaret Gutman.**] Distribution of phosphorus in the blood.

  2026. Further observations on the chemistry of cod liver oil.

  2140. [with **Margaret Gutman.**] The various forms of phosphoric acid in the blood.

  2141. [with **Marion Barnett.**] Observations on the distribution of anti-rachitic substances.

EXECUTIVE PROCEEDINGS.

One Hundred Twenty-fifth Meeting.

New York Post Graduate Medical College, October 18, 1922. President Wallace in the chair.

Members present: Baehr, Binger, Boots, Churchman, Cohen, Epstein, Greenwald, Hastings, Jackson, H. C., Kopeloff, Lundsgaard, Myers, Ottenberg, Rose, A. R., Schloss, Sherwin, Waksman, Wallace, Zucker.

Members elected: Wayne J. Atwell, Harry B. Eisberg, Frank A. Hartman, C. S. Robinson.

One Hundred Twenty-sixth Meeting.

University and Bellevue Hospital Medical College, November 18, 1922. President Wallace in the chair.

Members present: Bagg, Barber, Coleman, Eisberg, Friedman, Goldfarb, Greenwald, Hess, Hooper, Jackson, H. C., Killian, Kopeloff, Kleiner, Myers, Prewitt, Rose, A. R., Sherwin, Torrey, Wallace, Zucker.

Members elected: George E. Holm and DeWayne G. Richey.

A request was granted for the founding of a Peking (China) branch of the Society.

One Hundred Twenty-seventh Meeting.

Cornell University Medical College, December 20, 1922. President Wallace in the chair.

Members present: Baehr, Baumann, Chambers, Cohen, Dubin, Eddy, Funk, Hess, Jobling, Kopeloff, Myers, Ottenberg, Pohlman, Rose A. R., Rous, Senior, Sherwin, Shohl, Wallace, Zucker.

Members elected: E. F. Bostrom, O. W. H. Mitchell, Frank W. Weymouth, Charles E. Hayden, L. M. Hickernell, Robert K. Brewer, H. G. Weiskotten, Charles W. Young, Bernard E. Read, Charles W. Packard, Oswald H. Robertson, Henry E. Meleney,

Harvey J. Howard, Ernest C. Faust, Samuel B. Detwiler, E. E. H. Cruickshank, Hartley C. Embrey, Jui-heng Liu, Hsien Wu, Archibald McNeil, William E. Youland, Jr.

One Hundred Twenty-eighth Meeting.

College of Physicians and Surgeons, January 17, 1923. Vice President Jobling in the chair.

Members present: Baehr, DuBois, Eddy, Falk, Friedman, G. A., Goldfarb, Greenwald, Hastings, Jackson, H. C., Jobling, Myers, Noguchi, Pappenheimer, Ringer, Rose, A. R., Sherman, H. C., Williams, H. B., Wolbach, Zucker.

Members elected: David H. Dolley, Lester R. Dragstedt, James L. Gamble, Benjamin Roman, William Raymond Shannon.

One Hundred Twenty-ninth Meeting.

Presbyterian Hospital, February 21, 1923. President Wallace in the chair.

Members present: Bailey, Barr, Bostrom, Baumann, DuBois, Eisberg, Friedman, Greenwald, Harrop, Jobling, Killian, Lundsgaard, Mackenzie, MacNeal, Myers, Palmer, Riddle, Senior, Thomas, Wallace, Zucker.

Members elected: Lawrence B. Becking, George James Pierce, Wilbur W. Swingle, Frederick F. Tisdall, C. C. Young.

One Hundred Thirtieth Meeting.

Rockefeller Institute for Medical Research, March 21, 1923. President Wallace in the chair.

Members present: Bagg, Bailey, Barr, Bostrom, Boots, Davenport, Dubin, DuBois, Eisberg, Friedman, G. A., Funk, Greenwald, Gregory, Halsey, Hastings, Hatcher, Jackson, H. C., Kopeloff, Lynch, Metz, Myers, Prewitt, Rous, Thro, Van Slyke, Wallace, Zucker.

Members elected: Francis G. Blake, Ruth A. Guy, Frederick S. Hammett, Victor K. LaMer, Charles B. Lipman, Henry A. Murray, Grover F. Powers, Norris W. Rakestraw, Henry M. Ray, Florence R. Sabin, H. A. Spoehr, Edward S. Sundstroem, F. H. Sweet, Robert F. Loeb.

One Hundred Thirty-first Meeting. (Twentieth Annual Meeting.)

College of the City of New York, April 18, 1923. President Wallace in the chair.

Members present: Bailey, Baumann, Bergeim, Bostrom, Browne, Cecil, DuBois, Eisberg, Funk, Famulener, Gates, Goldfarb, Greenwald, Harris, Hess, Jackson, H. C., Jobling, Killian, Kleiner, Kopeloff, McNeal, Murphy, Myers, Pellini, Prewitt, Roman, Rose, A. R., Ringer, Scott, Sherman, H. C., Sherwin, Stillman, Thro, Uhlenhuth, Wallace.

Members elected: Henry M. Ray, Randolph West, Edgar G. Stillman, James W. Mavor, Henry R. Geyelin, Dana W. Atchley, Harald A. Salvesen.

Resignations: A. Carrel and L. L. Van Slyke.

Officers of the Society elected: Holmes C. Jackson, President; J. W. Jobling, Vice President; V. C. Myers, Secretary-Treasurer; S. R. Benedict, Member of Council.

Nominating Committee for 1924: A. J. Carlson, A. J. Goldfarb, L. B. Mendel, A. N. Richards, D. D. Van Slyke, George B. Wallace.

Endowment Fund: The Treasurer reported that through contributions made by members of the Society, the Meltzer Endowment Fund now amounts to $5500.00.

The annual dinner was held at the College of the City of New York following the one hundred thirty-first meeting. The following members were present: Bailey, Bergeim, Bostrom, Browne, Baumann, Cecil, Chambers, DuBois, Eisberg, Famulener, Funk, Friedman, Goldfarb, Greenwald, Harris, Hess, Jackson, H. C., Jobling, Kleiner, Kopeloff, Killian, Myers, MacNeal, Pappenheimer, Pellini, Prewitt, Ringer, Rose, A. R., Scott, G. G., Stillman, Sherwin, Thro, Uhlenhuth, Wallace, Zucker.

One Hundred Thirty-second Meeting.

Havemeyer Hall, Columbia University, May 16, 1923. President Wallace in the chair.

Members present: Avery, Berg, Bowman, L., Cole, Eddy, Edwards, Epstein, Falk, Famulener, Friedman, G. A., Funk, Greenwald, Harrop, Harrow, Jackson, H. C., Jobling, Killian, Mackenzie, Mann, Mueller, Myers, Oppenheimer, Ottenberg, Rose, A. R., Sherman, H. C., Thomas, Wallace, Winslow, Zucker.

Members elected: M. M. Brooks, Edward C. Rosenow, T. B. Magath, A. H. Sanford, C. S. Danzer, Edgar T. H. Tsen. Edgar D. Congdon, Carl F. Schmidt, George DeBord, Frederick Eberson.

Pacific Coast Branch.

Thirty-fourth Meeting.

Stanford University, October 14, 1922.
Members present: Alvarez, Alsberg, Walker, Lucas, Faber, Holmes, Fitzgerald, Beckwith, Hewlett, Swain, Martin, Barnett, Holman, Kofoid, Hanzlik, Dickson, Oliver, Manwaring, Schmidt.

Thirty-fifth Meeting.

Stanford Medical School, December 6, 1922.
Members present: Alsberg, Alvarez, Barnett, Clark, Dickson, Evans, Faber, Fitzgerald, Fleischner, Hanzlik, Hewlett, Holman, Kofoid, Langstroth, Lucas, Manwaring, Martin, Mehrtens, Schmidt, Smith, P. E., Towne, Walker, Weymouth.

Thirty-sixth Meeting.

University of California, San Francisco, February 14, 1923.
Members present: Alsberg, Alvarez, Barnett, Becking, Dickson, Evans, Holman, Langstroth, Lucas, Martin, Ophuls, Schmidt, Smith, P. E., Walker, Weymouth.

Thirty-seventh Meeting.

University of California, Berkeley, April 24, 1923.
Members present: Alvarez, Beckwith, Blatherwick, Clark, Dickson, Evans, Faber, Fitzgerald, Foster, Hall, Hanzlik, Holms, Langstroth, Lipman, Lucas, Martin, Mendel, Ophuls, Rakestraw, Schmidt, Sundstroem, Taylor, C. V., Walker.

MINNESOTA BRANCH.

Sixth Meeting.

University of Minnesota, Minneapolis, October 18, 1922.
Members present: Brown, Fahr, Fitch, Gortner, Henrici, Hirschfelder, Jackson, C. M., Kingsbury, Larson, Lund, McClendon, Palmer, Pettibone, Rasmussen, Scammon, Scott, F. H., Willaman.

Seventh Meeting.

University of Minnesota, Minneapolis, November 8, 1922.
Members present: Brown, Fahr, Gortner, Henrici, Hirschfelder, Jackson, C. M., Kingsbury, Lashley, Lund, Lyon, McClendon, Palmer, Pettibone, Rasmussen, Schultz, Scott, F. H.

Eighth Meeting.

University of Minnesota, Minneapolis, December 13, 1922.
Members present: Brown, Fahr, Henrici, Hirschfelder, Jackson, C. M., Kingsbury, Lund, Lyon, Scammon, Scott, F. H.

Ninth Meeting.

University of Minnesota, Minneapolis, January 10, 1923.
Members present: Fahr, Gortner, Hirschfelder, Larson, Lund, McClendon, Scott, F. H., Willaman.

Tenth Meeting

University of Minnesota, Minneapolis, February 14, 1923.
Members present: Lyon, McClendon, Henrici, Larson, Fahr.

Eleventh Meeting.

University of Minnesota, Minneapolis, March 14, 1923.
Members present: Hirschfelder, Kingsbury, Larson, Lund, Henrici, McClendon.

Twelfth Meeting.

University of Minnesota, Minneapolis, April 11, 1923.
Members present: Fahr, Gortner, Hirschfelder, Jackson, C. M., Kingsbury, Lund, Lyon, Palmer, Willaman.

Thirteenth Meeting.

University of Minnesota, Minneapolis, May 9, 1923.
Members present: Bell, Brown, Eckles, Fahr, Gortner, Hirschfelder, Jackson, C. M., Kingsbury, Larson, Lund, McClendon, Palmer, Scammon, Schutz, Scott, F. H.

Western New York Branch.

Third Meeting.

N. Y. State Agricultural Station, Geneva, October 14, 1922.
Members present: Anderson, Bloor, Clough, Dooley, Fish, Gaylord, Goldberg, Knowlton, Lathrop, Mellon, Murlin, Mattill, Simpson, Thatcher, Thomas, Williams.

Fourth Meeting.

Clifton Springs Sanitarium, N. Y., December 16, 1922.
Members present: Clough, Dooley, Fish, Goldberg, Hubbard, Mattill, Maynard, Mellon, Simpson, Thomas.

Fifth Meeting.

Syracuse University, New York, February 17, 1923.
Members present: Weiskotten, Hickernell, Brewer, Mitchell, Hayden, Simpson, Fish, Knowlton, Dooley.

Sixth Meeting.

University of Rochester, New York, April 14, 1923.
Members present: Anderson, Brewer, Clough, Fish, Hayden, Hubbard, Knowlton, Lathrop, Mattill, Mellon, Mitchell, Murlin, Simpson, Williams.

Seventh Meeting.

Cornell University, Ithaca, May 12, 1923.
Members present: Bloor, Brewer, Fish, Goldberg, Hayden, Hickernell, Hubbard, Mattill, Maynard, Mitchell, Murlin, Simpson, Weiskotten.

PEKING (CHINA) BRANCH.

First Meeting. (Organization.)

Peking Union Medical College, Peking, China, December 12, 1922.

Members present: Cruickshank, Faust, Howard, McLean, Packard, Read, Robertson, O. H., Ten Broeck, Wu.

Second Meeting.

Peking Union Medical College, Peking, China, March 7, 1923.
Members present: Meleney, Faust, Read, Schmidt, Ten Broeck.

REGISTER OF NAMES AND INSTITUTIONAL CONNECTION OF THE MEMBERS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE.

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OF THE

SCIENTIFIC PROCEEDINGS.

Addenda.

In Table I on page 254, the headings "$K_2C_2O_4$ present" and "$K_2C_2O_4$ not present" should be reversed.

E. V. McCOLLUM